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ON APPETITE JUICE AND THE ETHICS OF EATING

BY

J. G. ADAMI, M.A., M.D.

REPRINTED FROM THE MONTREAL MEDICAL JOURNAL, NOVEMBER, 1903.

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ON APPETITE JUICE AND THE ETHICS OF EATING.

BY

J. G. ADAMI, M.A., M.D.

Neat napery, food so served that its very look attracts, surroundings such that the mind is diverted for the nonce from the cares of the every day world,—cheer that, however simple, is before all things inviting,—this is the epitomized philosophy of Professor Pawlow,* of St. Petersburg, and by this philosophy would he teach us to exorcise the demon of dyspepsia. "Now, good digestion wait on appetite and health on both," he would say, had he known his Shakespeare, for what he insists upon is that good health depends upon good digestion and good digestion depends essentially upon appetite so that, for the preservation of health, appetite is to be cultivated. And upon appetite he has much to say.

His philosophy, let it be confessed, smacks of the epicurean. In these days of stress and strain, we are apt in the rush and turmoil of things, to make a virtue of necessity and taking no thought of what we eat or what we drink, impute this unto ourselves for righteousness. Far from emprelatising our entrails we are apt to glory in our neglect of creature needs. Whether we be strenuous or but merely neurotic, we are impatient that the flesh should and must obtrude; that it must obtrude is, we are inclined to hold, beneath our dignity as Men and Women. On the other hand, says Pawlow—we render his doctrine broadly:—"Take no thought for your food" is sound advice to those leading right and natural lives. The exercise of regular bodily labour and an out-of-doors existence, with an equable mind and a brain not overworked, conduce to hunger, and hunger is the best sauce. A genuinely hungry man can triumph over the matutinal hot roll and can accomplish the boarding house steak without serious after affects. An indoor existence, on the contrary, with little exercise and with food eaten as a matter of routine rather than in answer to the calls of hunger, and then with the mind occupied, not with the fare, but with the concerns of business, tends inevitably to dyspepsia. Under these conditions health can only be preserved if thought be taken of the food and if appetite be stimulated. And he proves this by a remarkable series of experiments.

* Pronounced, by-the-way, Pavloff, the *a* as in 'far.'

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Twenty years ago Pawlow was already embarked upon his studies on digestion. It is, I am almost shocked to think, verging upon that number of years since it was my good fortune to be a fellow worker with him at Breslau under the late Professor Heidenhain, in his time the foremost investigator upon secretion and the work of the digestive organs. Heidenhain was a master-mind and upon him Pawlow has built. Even then he was a marked man, serious, earnest, and quietly enthusiastic. Oddly, my most vivid memory of him is dietetic, for in their kindness towards the new comer in the laboratory, he and Madame Pawlow invited me to their celebration of old New Year's Eve, introducing me then to the Samovar and to Russian tea taken flavoured with rum and a slice of lemon,—a most delectable beverage on a winter's night, and the Silesian winter resembles the Canadian. But a few years later he was appointed Professor of Physiology in the Imperial Military Academy at St. Petersburg, and later was given, in addition, the directorship of the physiological department of the Institute for Experimental Medicine, an institute founded, magnificently appointed and endowed by Prince Alexander of Oldenburg, and there he possesses what is in every respect a model research laboratory. Steadily and without remission Pawlow has continued his studies upon the digestive system and, as the work has developed, he has attracted to himself a band of capable assistants—Chīgin, Hanicke, Katschowski, Kuwschinski, Kudreweski, Lobassow, Mdme. Shumow-Simanowski, Rjasanzew, Sokolow, Ssamoilow, Damaskin, Schepowalnikow, Wolkowitsch, Walther, Soborow, and a dozen others—who, under his direction, have undertaken an ordered succession of studies upon the different portions of the alimentary tract and its associated glandular organisms. The power that he has manifested to keep to one subject over this long period of time and of directing those under him in such a way that, without haste and without waste, the whole ground is gradually being covered, is remarkable. Remembering the similar year-long series of studies upon phagocytosis by Metchnikoff and his pupils, one wonders whether here we encounter a national Russian characteristic.

While through these investigations Pawlow's fame as a physiologist has as steadily increased, it has to be admitted that the medical world is only now beginning to recognize as it ought the great value of his work. In part, no doubt, this is due to the language in which many of the most important papers have been published; the Russian language is, for very many reasons, outside the pale. In part, however, it must be acknowledged, as physiology is becoming more and more specialised, that, as a body,—of course there are exceptions—physiologists nowadays pursue their science for science sake, without regard to its bearing as a

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branch of medical study, and as a result not one practitioner in a thousand subscribes to, or keeps himself posted regarding the contents of, the physiological journals. The greater number of the articles contained in those journals have little or no direct bearing upon the problems which immediately interest the ordinary medical man.

But Pawlow has throughout recognized the importance of his researches from a medical standpoint and, in 1897, he gave in St. Petersburg a series of lectures to medical men in which he brought together in a simple and entertaining form the outcome of the investigations made by him and his pupils. In 1902 he gave other lectures bringing the account of his work up to date. Translated into French and German, these lectures have had an immediate success. Now it is possible to read them in our own language, retranslated from the German by Professor Thompson, of Dublin.*

It is only a small book of less than 200 pages, but of these not a single one is uninteresting. So important is the work, so full of fact and thought and suggestion, that it should be in the hands, not only of every specialist upon digestive disorders, but of every physician. There has been nothing so valuable, nothing so fundamental upon the subject since Beaumont published his studies upon Alexis St. Martin. Upon these researches must be based the dietetics of the future.

Simply as a study in the experimental surgery of the digestive tract, the work is of importance. As a primary requisite for exact enquiry into the work of the different organs, it was essential to elaborate a technique whereby each gland or collection of glands could be isolated and its secretion obtained in a pure state over long periods of time and under varying conditions of experiment. It is unnecessary here to tell of the means whereby Pawlow and his co-workers successfully accomplished this in case after case; as an indication of the triumph over operative difficulties it may be stated that dogs have been kept for months in good health with the pancreatic duct opening upon the abdominal wall, that it has been found possible to keep dogs in relative health for many weeks when one vagus nerve after the other has been cut in the lower cervical region (thus cutting off the vagus innervation of the abdominal viscera), and that accessory stomachs have been moulded from the main organ, separated wholly from this as regards their contents while remaining still in vascular and nervous connection with the same. Such miniature stomachs secrete their juice *pari passu* with the main organ, so that samples of gastric juice secreted under different condi-

* **The Work of the Digestive Glands. Lectures by Professor J. P. Pawlow, translated into English by W. H. Thompson, M.D., King's Professor of the Institutes of Medicine, Trinity College, Dublin. London : Charles Griffin & Co., Limited. Philadelphia : J. B. Lippincott Company. 1902.**

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tions are to be obtained and analysed free from admixture with the food undergoing digestion in the latter.

With this perfected technique singularly accurate and constant results have been obtained. I shall not attempt to epitomize by any means all of them. Returning to my main thesis, I would take up the one subject of "appetite juice."

That there is a salivary appetite juice, is a well known and universal experience, the mouth watering in anticipation of food. That there is similar gastric and even pancreatic juice has not previously been accepted. If seen by experimenters, as for instance by those well known old observers, Bidder and Schmidt, it was noted to be inconstant; the laws governing the flow were neither suspected nor determined.

Employing œsophagotomised dogs, with gastric fistulæ and accessory stomachs, Pawlow has been able to show that several factors are at work determining the extent to which gastric juice is poured out in the act of digestion.

Take an œsophagotomised dog and mechanically irritate the mucous membrane of the mouth and throat, or feed it with smooth stones, the stones dropping out of the upper end of the œsophagus so soon as they are swallowed, and under these conditions, the stomach remains dry, not a drop of juice is excreted. Obviously the mere mechanical act of eating or of irritation of the throat does not reflexly induce an outflow of the gastric juice.

Make a gastric fistula in this dog and let it be hungry, with an empty stomach: not a drop of juice is present in the viscus; the mucosa is dry. This, it may be noted, was Beaumont's experience in the case of his French-Canadian voyageur. Nor does mechanical irritation, as with a feather, or even distension of the organ by means of a collapsible rubber ball lead to any secretion. The mere presence of matter filling the organ does not in itself excite the outflow of the gastric juice.

Take the same dog in a fairly hungry condition and begin to prepare and chop up meat before it in such a way that it does not imagine that it is being hoaxed, but anticipates the meal, then, in *five minutes'* time, there starts a rapid and relatively abundant flow of gastric juice.

Or give the œsophagotomised dog raw meat to eat; it gulps it down voraciously and, although never a particle reaches the stomach, five minutes again from the beginning of the meal there is a copious secretion of gastric juice, and this continues so long as the process of feeding is kept up.

In place of raw, give boiled meat; and the flow is not so extensive. If the animal has been fed only a few hours previously the flow may be

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slight. In short the amount of flow differs with different animals according to their state of hunger, according to the nature of the food, and according to personal idiosyncrasy. Some dogs have a stronger flow of juice, rich in pepsin, with raw meat than they have with bread, the meat is the more attractive. Others prefer bread to meat and, with it, have the more active secretion; some are phlegmatic and under all conditions give little of this appetite juice; others are sanguine and have generally an active flow.

Now, as a further exercise, take a dog having a gastric fistula and while it is asleep or while being awake, its attention is diverted, cautiously introduce into the stomach some pounded up raw flesh. Five, ten, fifteen, twenty minutes elapse and there is absolutely not a drop of juice discharged. It may be even an hour before there is any sign of secretion. More usually it begins in from twenty to thirty minutes. And here a long series of observations upon different food stuffs in animals with miniature stomachs and gastric fistulæ has proved that the different food stuffs act very differently as *direct* excitants of the gastric secretion. The results, in fact, are largely unexpected. Simple proteids like white of egg or like meat that has been boiled for days to remove all extractive matters, are wholly inert. They may lie in the stomach for hours (when directly introduced) without there being any outflow. Starches are likewise inert. Sodium bicarbonate and common salt are found, contrary to all previous teaching, when given with other substances, to lessen the outflow. Fats also have an inhibitory effect, but water in fair quantities directly incites a flow and so do meat broths and various extracts of meat.

We have, that is, to deal with two tides of secretion of the gastric juice; the psychic tide, set up by the sight of food, by the taste of the same, and by hunger, and the juice of this tide—the appetite juice—is relatively abundant, flows for a considerable period, and has strong digestive powers. And secondly, the chemical tide, set up at a later period by the direct effect of the food stuffs upon the gastric mucous membrane. The former is brought into activity by the higher centres. Cut through the vagus nerves and it is wholly stopped. The latter has also, according to Pawlow, a nervous origin being brought about by the simple reflex from irritation or chemical stimulation of the gastric mucosa. Taking everything into consideration, the former tide, of "appetite juice," is by far the more important. I may here quote one of Pawlow's own experiments amply proving this statement.

"I carried out the following procedures upon two dogs both of which had ordinary gastric fistulæ and were besides œsophagotomised. Into the stomach of the one, while its attention was distracted by patting

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and speaking kindly to it in order to avoid arousing any thoughts of feeding, a definite number of pieces of flesh were introduced through the fistula. The morsels were threaded on a string, the free end of which was fastened to the fistular cannula by inserting a cork. The dog was then brought into a separate room and left to itself. A like number of pieces was introduced into the stomach of the other dog in the same way, but during the process, a vigorous sham feeding was kept up, the animal being afterwards left alone. Each dog received 100 grms. of flesh. Since then an hour and a half have elapsed, and now we may draw the pieces of flesh out by means of the thread and weigh them. The loss of weight, and consequently the amount of flesh digested, is very different in the two cases. In that of the dog without sham feeding, the loss of weight amounts to merely 6 grms., while the flesh withdrawn from the stomach of the other dog weighs only 70 grms., that is to say, has been reduced by 30 grms. This, therefore, represents the digestive value of the passage of food through the mouth, the value of an eager desire for food; the value of an appetite."

"A good appetite in eating is equivalent from the outset to a vigorous secretion of the strongest juice; where there is no appetite this juice is also absent. To restore appetite to a man means to secure him a large stock of gastric juice wherewith to begin the digestion of the meal."

The morals are many which we may draw from this striking series of experiments. First and foremost, we have to learn that, in dieting either the well or the ill, the absolute quantity of the food stuffs we give and the number of calories these quantities represent, is far from being everything. A simple food stuff like white of egg, if given alone, will take a long time for its digestion, whereas a simple broth or extract of meat, whose direct value as a food is absolutely nil, may be of distinct value in directly stimulating the flow of the gastric juice and thus aiding the digestion of food stuffs which follow. Plain undiluted water has a like affect. Food eaten without relish and without appetite, although in itself most nutritious, may lie for hours within the stomach undigested. So again, food eaten while the mind is diverted to other things, may stay for long unacted upon, nay more, under such conditions, is liable to undergo decomposition and, irritating the mucous membrane, may lower its condition. Here, in short, appears to be the basis of the dyspepsia so common here as to be *the* American disease. I do not mean to say that bad cooking, iced drinks and creams, hot rolls, and so on, do not play their part, but even granting all these things, if we are to accept the abundant experiments of Pawlow and his

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co-workers, the conditions under which the food is eaten are the main causes of the disorder. "The old and empirical requirement that food should be eaten with interest and enjoyment is the most imperatively emphasized and strengthened of all." And if dyspepsia is to be warded off, the food should be taken under such conditions that everything is directed on the one hand to remove the thoughts from the cares of daily life and, on the other hand, to make the repast appetising so that the palate may be tickled and the flow of appetite juice excited. No matter how good the food, if presented, as is too often the case in our hotels, our public institutions, our hospitals and even in our homes, in an unattractive form, then, the appetite is not awakened, the appetite juice flows but poorly, and the digestive process is delayed to the detriment of the organism and of the individual, and of his mental and moral tone.

There is thus a philosophical basis for the customs of the table and for the decent carrying forward of the meal. Even, as Pawlow indicates, a good word has to be said for the preliminary cocktail, or glass of sherry and bitters. For, where food has to be taken and the individual is not absolutely anhungered, the bitters act as stimulating the appetite, that is, as preparing the organism to be attracted by food. Wholly apart from the question of the food value of alcohol, he is apt to consider that, at times, a little wine with the meal is a benefit in that it produces a mild narcosis of the highest centres and so distracts the mind from its cares while not acting upon the higher centres of the second order which determine appetite. The *hors d'œuvre*, it is to be noted, formed of highly seasoned fish or fish paste or the like, and rich in extractives, both stimulates the appetite and has a direct affect upon the gastric mucosa. Soup again, having a basis of stock or meat broth, comes well at the beginning of the meal in that the extractives it contains and the water directly stimulate the flow of the gastric juice and so prepare the stomach for the more solid foods that follow. While "the repast begun with pleasure consequent upon the pressing need for food, must also, notwithstanding the stilling of hunger, be terminated with an agreeable sensation. At the same time the digestive canal must not be burdened with work at this stage. It is only the gustatory nerves which should be agreeably excited; hence the logic of ending with something sweet."

It must ever be kept in mind that "what is meat for one is poison for another," that the same diet, found nutritious for one, is eminently unlikely to be serviceable for every individual, but that, on the contrary, in dieting, the personal equation must be carefully studied and those dishes be given which the patient enjoys, even if the food value, as

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reckoned outside the body, of some of the ingredients, be far from approaching that of the constituents of other foods for which the patient has no appetite. And, remembering what has already been said concerning the pouring out of appetite juice, it is clear that if we wish to feed up a patient with a weak digestion, it is better not to give large meals but smaller meals at shorter intervals, for, with each meal, the flow of appetite juice is called into activity.

But for other points here touched upon and their explanation, reference has to be made to Pawlow's own work. There is one matter which I do not see touched upon by Professor Pawlow, namely, how far we are justified in drawing deductions from these experiments upon the dog regarding what occurs in man. If, as Pawlow puts it, this flow of appetite juice is brought about primarily by the intervention of higher functions such as judgment, will, desire, and if, by the establishment of this passionate desire for eating, unerring and untiring nature has linked the seeking and finding of food with the commencement of the work of digestion, then it has to be realized that the desire for special forms of food in a carnivorous animal must differ from the desire and choice of an omnivorous animal. So that, while flesh, more particularly, excites the dog, it may be that bread and other starchy foods and even sweet stuffs have in man an even stronger stimulating affect. To this extent we must be prepared to find differences in details in the working of the human as compared with the canine stomach.

Can we go any further? I think it will have to be admitted that we can, and that there are other differences between the flow in man and in the dog. I have just, as a matter of interest, been reading that which was the first great American contribution to medical science, William Beaumont's "Experiments and Observations on the Gastric Juice and the Physiology of Digestion," of which the first edition, which, along with the second, we are fortunate in having in our library at McGill, was printed in "Plattsburgh" in 1833. You all know the main points of the story of the French Canadian, Alexis St. Martin, how in his youth he was accidentally shot through the body, leaving a permanent gastric fistula which, nevertheless, caused him so little eventual serious disturbance that he lived to be eighty-one, and you know how, on and off for very many years, Beaumont, the army surgeon, made observations upon him. If you wish to refresh your memories as to the details of this most interesting history, I need but refer you to Dr. Osler's paper in the Journal of the American Medical Association of November the 15th, 1902.

Reading the original, one is immediately struck with a very marked difference between Pawlow's results upon the dog and Beaumont's upon

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St. Martin. You will remember that the former found that mere mechanical stimulation of the gastric mucosa did not, in itself, call forth any flow of the gastric juice. Beaumont, on the contrary, employed this method of mechanical stimulation as a routine practice in order to obtain the pure juice. His method of gaining such juice, indeed, was simply the introduction of a gum elastic tube of the size of a large quill through the fistula into the stomach. "In health," he says, "and when free from food the stomach is usually entirely empty and contracted upon itself. On introducing a tube, the fluid soon begins to flow, first in drops then in an interrupted, and sometimes in a short continuous stream. Moving the tube up and down, or backwards and forwards, increases the discharge." Now this conclusion was not reached as the result of an occasional observation. There are, I have counted, some three score observations detailed in which studies were made with juice so gained from the fasting stomach. Nor was it a mere watery mucoid discharge that was gained by this means; it was a typical gastric juice, capable of acting upon various meats, milk, white of egg, and a host of other substances; it was strong enough to act upon bone; it was analyzed and hydrochloric acid was found present. It is true that ten, fifteen, or more minutes were necessary to collect even one and a half ounces of juice by this mode of irritation of the healthy, fasting stomach, so that the flow so induced was not found very active, true also that, occasionally no fluid was obtained by this means. But Beaumont suggests, what is at least a reasonable explanation, namely, that the irritation so induced is localised and not general, so the amount of secretion was not great. As Beaumont calls to mind, Spallanzani and others had, before him, excited the discharge of the gastric juice by the introduction of such inert substances as sponge, tubes, pebbles, etc., into the stomach *per os*. I do not see, therefore, how we can possibly accept Pawlow's generalization that the human stomach is not stimulated to secrete juice by mechanical irritation. There is a difference between man and his more highly organized nervous system and the dog. The human stomach is the more sensitive. Admitting this, we obtain an explanation of an apparent paradox which Pawlow points out and attempts imperfectly to explain. He gives an instance from his own personal experience in which, after an illness accompanied by a transient high fever, he had lost all desire for food and had a complete indifference towards eating. Fearing collapse, on the second or the third day, he endeavoured to create an appetite by swallowing a mouthful of wine. He felt is quite distinctly pass along the œsophagus and into the stomach and literally, at that moment, perceived the onset of a strong

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appetite. He admits, therefore, that the tactile sensation of the stomach is, in man, sufficiently developed to be, at times, recognized by the psychical centres and, if he admits this, then it appears to me that he must go one step further and admit that, in the human being, such sensation is capable of starting the reflex which leads to a secretion of the juice. So far as they go, the facts here recorded seem to establish the distinction between man and the dog. One possibility remains, namely, that St. Martin knew what was expected of him and that the flow was stimulated from the higher centres and was not a simple mechanical reflex. Beaumont never tried the effect of introducing substances when the attention was diverted, and his observations show that in the middle of the experiments when his subject had became accustomed to the procedure, the flow was often very small, only a drachm or two and that, rarely, it was completely wanting. Granting this, I cannot but think that the evidence is in favour of the human stomach recognizing the existence of a mechanical load and secreting accordingly. If this be so, I do not see that we can hope ever to obtain in man so perfect a demonstration of the different tides of the gastric juice as Pawlow obtained in the dog. But, with this exception, it seems that we must regard Pawlow's observations as very largely applicable. Beaumont, it is true, only made what we may term rudimentary observations. He did not—he could not—estimate the power of the gastric juice under different conditions. He did not make sufficiently accurate observations upon the digestion of individual constituents of the diet; if he gave eggs, it was the whole egg together with salt and generally with bread. He experimented upon an enormous number of foods, even down to soused pig's feet and apple dumplings. He gave these with coffee, bread and vegetables. But, like the later observers, he found, under ordinary conditions, that within fifteen minutes the juice was already being actively discharged, and the discharge was largely proportional to the food taken, and that emotions and "whatever depresses or disturbs the nervous system" arrest the flow. I do not see that we can fail to be convinced that the broad principles of the two orders of excitation, psychic and chemical, must be the same in all warm blooded animals. What rests with us is, to observe carefully the effects of accepting Pawlow's principles in our dieting of the individual. Thus far it may be laid down as a matter of experience that the most well-fed persons are those who feed best.

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ADAPTATION AND TUBERCULOSIS.[1]

BY

J. G. ADAMI, M.A., M.D., F.R.S.,

of Montreal, Can.

McGill University, Montreal.

In casting around for some aspect of the tuberculosis problem upon which to address this meeting, it struck me that it might be serviceable to take up the matter of adaptation in its relationship to the disease. The term possibly is unfamiliar to you, but it embraces a series of processes, both on the part of the organism—the human body—and of the microorganism—the tubercle bacillus—which are of the highest importance. And I am of the opinion that it is the failure to realize the existence of these processes which renders it difficult for the majority of men to appreciate the various happenings in the course of this disease, and again the points at issue and their significance in the controversies that have arisen of late years regarding the same. It has seemed to me that all those, and they are many, who are interested in the work of prevention, would possess a more intimate appreciation of that work if they could acquire, as it were, a mental picture of the moves in the game, of those moves whereby now the organism, now the microorganism seeks to gain the advantage and checkmate the other. In truth, it is a gruesome game, but one of most vital import, this of the cells against the bacilli and the bacilli against the cells. Some of our moves are instinctive, or have been practised before against other bacteria; many have to be learned and tested during the course of play. Too often, not knowing the science of the game, and playing "Bumble puppy" (I forget its equivalent in chess) we make a wrong move at a critical moment, and the game is lost—and loss is death.

Let us consider first the moves on the part of the organism, and in order to gain a clearer picture let us take the case of a disease of briefer course and appar-

[1] An address delivered at the annual meeting of the Dominion Association for the Prevention of Tuberculosis, Ottawa, March 15, 1905.

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ently more self-contained in its gross effects upon the body. Has it ever struck you, I wonder, what takes place in a case of acute pneumonia? This, as you know, is a bacterial disease, due to a micrococcus, a minute rounded or lance-head shaped organism that hunts, if I may so express it, like the Irish Constabulary or the Northwest Mounted Police of the old days, in pairs—hence we often speak of it as the diplococcus of pneumonia. You all know the disease develops very rapidly. That rapid development is associated with an extraordinarily rapid multiplication of the diplococci, so that these, which under ordinary circumstances are not present in the lung, come to be present in teeming millions in the air sacs of the same, and there by their poisons set up so much irritation that all the air sacs of one or more lobes of the lung become solid through the intense inflammatory exudate that is poured out into them from the bloodvessels, displacing the air that should be there. So it is that in a very few hours the affected part of the lung comes to look more like a piece of liver than like a sponge with abundant air in its cavities. Then, if all goes well, in four, six or eight days, suddenly, in the course of a few hours the crisis comes, the fever drops sharply, the patient feels better, and after this crisis we find the diplococci for the most part dead, or if not dead, so weakened that they can have little effect on small animals.

This has always seemed to me as something approaching the miraculous, that bacteria grow abundantly in one of the tissues of the body for a few days, then as suddenly they are killed off and disappear. If they grow at first, why do they not continue to grow? if eventually killed, why not killed at the start? Years ago we found out that this was not because they had exhausted the food supply. I do not know if this has been tried in connection with pneumonia, but it has been repeatedly tested in the lower animals in connection with one or other of the diseases from which these may suffer; it has been found that the blood and tissues of the diseased animal will afford abundant nutriment for the bacteria. It is not that they are poisoned by the products of their own growth—this occurs, it is true, when we grow them on broth in a closed test-tube outside the body—but we can make an emulsion of a pneumonic lung, and, while if we add the diplococci to this, some will be killed (for, as I shall point out, there are substances poisonous to the bacteria in such a lung), yet when a certain number have

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been killed the rest will grow freely. If the poisons were produced by the diplococci themselves then the greater the number of bacteria destroyed the more of these poisons would be liberated into the lung emulsion and still less the chance would be for any to remain alive and multiply.

Neither of these explanations will suffice. The only adequate explanation for this eventual destruction of the bacteria is that of adaptation. When first the diplococci began to grow in the lung they did so because the tissues could not neutralize their poisons and kill them; by the very act of growth and production of poisons on the part of the bacteria the tissues become educated until the moment is reached when the cells of the body produce sufficient counteracting poison to kill off the bacteria and to neutralize their toxins, which toxins do all the damage to the system.

And here is the interesting and important fact—a fact, I think, too little realized by most medical men, although instinctively all strive to act up to it. It is not the lungs alone that are in action in destroying the germs of the disease and so bringing about recovery, it is not even the white corpuscles or leukocytes which, passing into the lungs, accomplish the good effects; the whole organism, or practically the whole organism, is actively engaged in the process.

You have all, I doubt not, heard much of late years about these white blood-corpuscles or leukocytes; how they are, as it were, at once the main avenging army and the scavengers of the body; without doubt these play a great part. We can see them in various stages full of bacteria which they have taken up and at times we can make out that these bacteria are undergoing digestion and destruction. Nay, it is not difficult for anyone to experiment on himself, as Leishman has shown, to take a few drops of his own blood, separate off the white blood-corpuscles and taking a drop of blood-serum, holding these in suspension, add to it a number of disease-producing bacteria of one or other order; in 15 minutes time, kept at the body temperature, each little leukocyte can be seen to have taken up, it may be a score or more separate bacteria. But here is another fact. This eating up of the bacteria does not depend upon the white blood-corpuscles alone. It depends, as Wright and Douglas have shown recently, upon a curious interaction between the cells and the fluid of the blood. If you take the white corpuscles of a man who has not had a

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given disease and place some in the fluid of his own blood, and place some others in the blood-serum of a man who has successfully resisted that same disease—who has recovered from an attack—you will find that these little white blood-corpuscles will take up very many more of the particular bacteria causing that disease in the latter case than in the former. There is, therefore, something circulating in the general fluids of the body after it has gone through an infective disease, something not local but generalized, something which was not there before in any amount and has therefore been elaborated during the course of the disease, and this aids in the destruction of the bacteria of the disease.

The same was noted some years ago in connection with typhoid fever and that not merely after recovery but during the process of the disease. We utilize the fact now as a most useful means in diagnosing doubtful cases. In typhoid the bacilli grow more particularly in the lymphoid tissue of the intestine—for here is an interesting point to remember, that the different bacteria of disease have their seats of election. Once they gain a footing in the body there are certain tissues in which they grow in greatest abundance, while at first they do not grow to any extent in other tissues; or, in other words, if they do find their way into the other tissues they are easily destroyed. But while the typhoid bacilli thus grow locally if we take the fluid of the blood of a typhoid fever patient on the fifth day of the disease or so, we find that this now has new or greatly exalted properties. Although we dilute that blood 40 or 50 times, if we place in it some of the actively growing typhoid bacilli they become motionless and clump together in masses. The blood fluid has acted upon them. Nothing of the kind occurs in similarly diluted blood from one who has not had typhoid. What does this all mean? It means that during the course of the disease there is gradually developed on the part of the organism as a whole the power of coping with and neutralizing or destroying the microorganisms of that disease. Something has developed, not locally, but generally, which either was not there before or which now is developed in greater quantity than before. There is an adaptation to changed conditions. The body as a whole reacts and produces substances which tend to give it the advantage in the fight against its foes.

Need I remind you that the modern treatment of diphtheria makes use of this fact. We employ the fluid

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of the blood of animals which have been inoculated with the diphtheria bacillus, in order to utilize the antitoxic substances which those animals have produced, and produced in excess, in order to cope with the inoculated microbes. Where precisely these antitoxic substances are produced, we are still engaged in determining. We know that the leukocytes produce one set, but the substance or substances which activate these and render them effective, we know less about. Some are inclined to believe that the leukocytes also give origin to these. Recent evidence tends to show that certain tissue cells—of the liver, brain, etc.—elaborate them. It may well be that in tuberculosis the muscle cells play some part.

So now to return to the case of pneumonia. Let us try to translate what happens there. Through some lowering of vitality, the tissues of the air sacs, which in health can destroy individual bacilli finding an entrance into the lungs, are overcome, and the bacteria multiply and set up disturbance. Then the second line of defense comes into action—not so much the lung tissue itself as the leukocytes which belong to the general circulating blood. These make their way into the damaged area, are unable to take up diplococci in sufficient numbers and destroy them; on the contrary, they themselves tend to be destroyed, and the diplococci continue to multiply. In the meantime, the poisons from the diplococci have diffused out of the air sacs into the blood and so are carried all over the body, and with this we have the development of high fever. And now the cells of other parts of the body take up these less concentrated poisons or toxins, and taking them up, proceed to manufacture the counteracting bodies which neutralize, or help in the process of neutralization of the poisons, and once they start to do this, they continue, and produce more and more of these antitoxic bodies, so much in fact that the excess passes into the blood, and from the blood passes into the damaged lungs, until the moment is at last reached, when sufficient of these antitoxic bodies are present there to reinforce the action of the leukocytes, and with this, all the diplococci are killed and recovery ensues. I say reinforce the leukocytes, for the leukocytes are developed largely in the marrow of the bones, and the later relays of young leukocytes have, before they reach the lungs, become accustomed and adapted to the bacterial poisons, and thus are much more powerful than the earlier drafts of leukocytes which passed into the lungs. These, aided by the

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fluid of the blood, are effective, the former were not. Hence, it is through the general adaptation of the tissues of the organism, and not merely through local efforts, that the body overcomes infective disease.

Once one realizes this it is all so clear, and, if I may so express it, so very human—so like, for example, what happened in the Boer war. There we had local irritation in one part of that vast organism, the Empire; local efforts were unable to quell the disturbance, and war flared up and there was great local damage and arrest of the normal local activities. It looked as though the part might be completely lost. The effects of this local disturbance rapidly diffused through and influenced the whole empire, and, like the leukocytes, soldiers were drafted to the seat of irritation from all parts of the organism, even from distant portions like our own Canada. We contributed, as it were, from the marrow of one of the limbs. Those soldiers, at first unused to Boer methods of warfare, were at a great disadvantage and we had Nicholson's Nek and Colenso and other terrible diasters. But as the Boer methods became better understood our soldiers adapted themselves to them; the spirit of depression gave way to one of grim determination to overcome the enemy; more and more soldiers, contingent after contingent, from all parts of the empire were collected and sent to the front. Supplies of all kinds were produced at a distance and poured into the focus of inflammation, and at last the pathogenic organisms were completely overwhelmed and recovery ensued.

Now to apply all this to tuberculosis and its arrest. The disease, it is true, is of a different type—it is of slower development and more progressive character. To pursue my simile, if I may venture to do so without offence and without wishing to give offence, tuberculosis is to the human organism something like what Irish discontent is to the body politic. If we are healthy our first line of defense, the surface cells of the nose, mouth, throat, air passages, and digestive tract, can directly destroy occasional tubercle bacilli taken up by them, only if an excessive number be taken up are they killed by the bacilli. Healthy people can breathe in tubercle bacilli without harm resulting. That this occurs has been proved by examining the nasal secretions of nurses and students in tuberculosis wards and finding tubercle bacilli in the same, and I may point out the remarkable fact that in a well-conducted tuberculosis

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hospital the nurses are found not to contract tuberculosis. They keep themselves in good condition.

The bacilli may get beyond this first line of defense into the lymph and blood, and there may not cause any disturbance, being killed before they can multiply. Quite a number of cases are on record in which tubercle bacilli have been found in apparently healthy lymph-glands showing no signs of tuberculosis. Again we can, for example, take two healthy young dogs and feed them with milk to which we have added a fair but not excessive number of active tubercle bacilli, and killing one two or three hours later, we can detect the tubercle bacilli in the lymphatic fluid draining away from the intestines. This is a process which, as I and others have pointed out, is constantly proceeding to a slight extent in connection with the abundant bacteria of various kinds which people the intestines. Keeping the other dog for some weeks or months, it may show not a sign of tuberculosis, and killing it at the end of this time we may not detect a sign of this disease in any region of its body.

But now if even temporarily the general health is depressed the history may be very different. The tubercle bacilli at the point of entrance, or it may be when they are carried into the circulating lymph or blood, are not necessarily destroyed. In many parts of the body they are, but if an organism possesses an Ireland—a region of constitutional weakness, with poor nutrition and poor circulation—if by chance the bacilli find their way into this, the cells cannot destroy them, but, on the contrary, they multiply, produce their poisons, killing the cells, and developing a focus of inflammation—a tubercle. Such a region, as everyone knows, is the apical part of either lung. From its relationship to other parts, there is there poor circulation and nutrition, and, added to this—although here remembering my simile, I must speak delicately—there may be something innate in the properties of the tissue cells themselves. Certain it is that here more particularly the tuberculous process may manifest itself. A priori, one would think that the bacilli having once gained a footing in a part would continue to grow and spread from this focus; that growing their concentrated toxins would depress the vitality of surrounding cells, rendering them an easy prey, so that of necessity once the disease was established in the system, it would go on from bad to worse with progressive invasion, poisoning and destruction of the tissues

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throughout the body until a merciful death ended the scene. This does occur in some cases in which the tissues seem to have no resisting power, but as a matter of fact, it is by no means necessarily or usually the case. Progressive invasion we know is the exception, not the rule. As a matter of interest, I looked last week through the records of the 139 postmortem examinations performed last year in my department at the Royal Victoria Hospital, and I found that while there were 18 cases out of the total in which tuberculosis had assumed a progressive character, and had surely been the cause of death, there were 41 cases, or more than twice as many, in which there were absolute evidences of old arrested, and even healed tuberculosis. There were, in addition, three cases of progressive tuberculosis in which death was from some other cause. The disease, as has been often stated before, is more often arrested in man than it is fatal, and the process in this arrest and healing must from every consideration be not so much by local effort as by the cooperation of the other tissues. We have clear evidence that this is so. Just as in typhoid fever so here it has been shown, more particularly by Courmont, that the blood and body fluids of tuberculous patients contain a substance not present in healthy blood, a substance which causes the clumping of the tubercle bacilli. And, as pointed out long ago by Koch, if an animal has localized tuberculous affection of one region, say the eye, the injection of virulent tubercle bacilli into another region at a distance, say the skin of the flank, leads, it is true to a temporary local inflammation during which the bacilli are destroyed, but it is followed by no local development of the disease proper, and by no extension from that region; a clear proof that under ordinary conditions the primary local development of the disease is accompanied by the development of increased resisting powers on the part of the rest of the tissues. Here again there is adaptation, by means of which these other tissues of the organism protect themselves, and as a whole reinforce a local effort, tending to produce so much antitoxic or antibacterial substance that at last the system overwhelms and arrests the local growth of the bacilli.

I have not seen this matter hitherto worked out adequately, and I believe it is useful to present it to those interested in our work, even though at first hearing—being perhaps to some extent novel—it may be difficult to follow and fully grasp. Once grasped we

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grasp with it the whole rationale of the treatment of tuberculosis. Let us just glance at this.

First as to Koch's treatment by injections of tuberculin; that is, of the body juices and toxins of the tubercle bacilli. The basis of this treatment is clearly the carrying further of this natural process of stimulating the tissues in general to produce antibacterial substances by means of the circulating toxins. As we know by observation outside the body the individual tubercle bacilli do not produce much toxin; indeed, it is only when they die or are destroyed that much poison escapes from them. Probably one of the reasons why tuberculosis tends to gain a foothold in the body is that the bacilli are at once so slightly irritant and so resistant. As there is no extensive diffusion of toxins at the beginning of the process, the rest of the tissues are not adequately stimulated; this especially when the body as a whole is in a low state of nutrition. By injecting these diffusible toxins we stimulate the cells in general to manufacture increased amounts of antitoxic substance and thus aid the local resistance. [I put this purposely in a general way; to discuss this matter in the terms of complements and amboceptors and all the armamentarium of the modern bacteriologist, would utterly confuse, but this obviously is at base the rationale of the process.]

But as all now know, Koch's treatment is but partially successful. It is useless in advanced cases in which the disease is extensive and in which there must already be relatively abundant circulating toxins. To inject more toxins in such cases is to poison rather than to stimulate the cells. In dealing with the treatment of tuberculosis there are two factors to be taken into account: You may take a horse to the water, but you cannot make him drink. You may supply a cell with the tubercle toxins which are necessary in order to stimulate it to produce antitoxins, but it may be so feeble that it will not react —will not produce these toxins. All its energies may be used up in the performance of ordinary everyday functions. And here we have the basis of the modern treatment in which, as you know, we do not try to do anything specifically against the disease itself; on the contrary, we leave the disease as such severely alone. But we do everything in our power to improve the general bodily condition. We enforce rest so that the cells shall not be overworked, and may have spare energy; we give abundant, easily assimilable food so that they

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may build themselves up; we demand life in the open air with abundant oxygen, and that toning up of the system which the freshness and coolness in the air bring about more naturally than does anything else. For just as a lax violin string will give no note, while made taut it vibrates to the slightest touch, so by improving the tone of the tissues in general, they respond more immediately and more fully to the stimulus of the circulating toxins, and produce the counteracting bodies which, developed in greater abundance and poured out into the blood, can now act locally on the tubercle bacilli in the areas of disease.

We in short do everything we can to help the body adapt itself to the changed conditions and this adaptation we know means also counteraction. The success of our modern treatment of tuberculosis—treatment, be it remarked, purely empiric in its inception and based upon no adequate theory of the modes of defense on the part of the organism—this success is the strongest proof of the correctness of the conclusion reached along other lines, that recovery from infective disease is not merely nor mainly a local reaction, but is a process in which the tissues not directly involved and the body as a whole take a most active part; becoming educated thereto during the course of the disease.

I have taken possibly too much of your time in discussing the moves on the part of the organism and have delved, it may be, too deeply for a general address. I would gladly think that my digging, if deep, has also been sufficiently broad in its scope to let in the light. Before closing, some words must be said of that other matter, the moves made by the bacilli.

You must not look upon these producers of disease as fixed in their properties and unalterable; rather we have to realize that they also are capable of adaptation. For us it is a fortunate fact that their power of adaptation is not so extensive and so rapidly developed as that of the healthy human organism. This we may take as another instance of the fact that union is strength. It may well be that the individual cells of the body have not the same power of adaptation as has the tubercle bacillus, but while the bacilli are isolated and independent the cells of the body are united and cooperate and the sum of their reactive changes may well be greater than the adaptative changes possible in an isolated tubercle bacillus. Nevertheless, bacteria are capable of great changes, suiting them to altered conditions

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of their surroundings. There is, for example, a large bacillus, *Bacillus megatherium*, first found, if I remember aright, upon a cabbage; this is absolutely harmless for warm-blooded animals—one can inject it by the million into the rabbit without setting up any recognizable disturbance—but, as Vincent has pointed out, place some of these in a thin-walled celloidin capsule in the abdominal cavity of the rabbit, such a capsule that the fluid part of the lymph can easily penetrate through the walls and so afford nourishment to the bacilli while the leukocytes and antitoxic bodies cannot enter—we find that after sojourning there for several weeks the bacilli have become accustomed to their surroundings so that now they will grow in the tissues of the rabbit without any capsule being needed. From having been perfectly harmless they are now pathogenic and can set up disease.

What is to be said regarding the tubercle bacillus in this connection? In the first place, we may have the complete assurance that Adam was not created suffering from tuberculosis. The bacillus, we may be fairly sure, from living it may be on foodstuffs outside the body, accustomed itself first to living on the surface and in the passages of the organism as a harmless saprophyte and only later gained the power of living not on, but in the tissues, and from that moment it became pathogenic. This, it is true, must have happened centuries and centuries ago, for the disease was known to and well described by the early Greek writers on medicine. While this is so, I do not think that we must imagine that the virulence of the bacillus has remained the same from that day to this; the probability is that were the ancient Greek to come to life again and mingle with us moderns his would be but a brief visit to this earth, he would be carried off by fulminating malignant tuberculosis in a very short space of time, if even before that the modern influenza bacillus or the pneumonia diplococcus had not marked him for its own. I mean that, the indications are that there has been a steady adaptation of both organism and microorganism, the one to the other; as the system has become more resistant, the bacillus has become more toxic. We have a parallel to what is here suggested in the remarkable history of the way in which the South Sea Islands were devastated by measles when that most puerile disease was first introduced by Europeans. We must suppose that measles had originated in Europe and Asia at some period after

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the first natives found their way across to the South Sea Islands, or that the colonizing aborigines did not happen to carry it with them in their canoes and so henceforth remained free. Probably it began as a mild disease and as it became habituated to the human organism so did that organism become more resistant, and the microbe increase in virulence *pari passu;* what continued to be a mild disease to Europeans therefore was most fatal to the Melanesians who had not undergone this progressive adaptation.

We have abundant evidence bearing upon this matter of modification in the virulence of bacilli by growth in the organism of one or other species; adaptation that is, to the surroundings whereby existence is rendered more sure. By the passage of a given pathogenic bacillus through a given series of animals, by inoculating one animal of a species—a guineapig for example—with a feebly pathogenic microbe, then when the disease is in full activity taking some of the body fluids containing the germs and inoculating that into another guineapig, and from this again into another, and so on through a succession of a score or so—we can render the bacilli extraordinarily virulent, so that whereas the disease in the first of the series ended in natural cure, at the end of the series the greatly diluted body fluids, diluted so as to contain only a few rare microbes when injected, may cause death in from six to ten hours.

By this artificial process, bacteria adapt, and more than adapt, themselves to the organism of the one particular species; but this does not necessarily mean that they have adapted themselves at the same time to the conditions found in the organisms of other species. That may or may not be the case. An organism which, by passage through a series of human beings has acquired greater virulence for man, may or may not gain increased virulence, say for oxen, and vice versa. On the whole, the reverse is more often the case. As a matter of fact, we have positive evidence that if we take two calves and inoculate them subcutaneously with equal amounts of cultures of tubercle bacilli, which have been gained from the cow and man respectively, the disease is much more rapid in its progress, spreads much more rapidly, and leads to earlier death when the bovine bacillus is employed than when the human strain has been used. This may be laid down as a general rule. Nay, more; if only a moderate dose of bacilli gained from man be injected, nothing more than a

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local nodule is produced in the inoculated calf; there is no generalization, and after a few weeks or months no signs of the tubercle bacilli are to be made out.[1] So much so is this the case that von Behring is now utilizing bacilli gained from cases of human tuberculosis, to vaccinate cattle and to prevent them from becoming infected from their fellows by means of the bovine tubercle bacilli. This is all now freely accepted; the opposite case remains still a matter of some debate, though the two parties are coming to take a more intermediate position. This matter was discussed very thoroughly by Dr. Ravenel in the address before this Association last year, and Dr. Ravenel, you may remember, took the position that tuberculosis is rather frequently conveyed to man from cattle. I still hold that such conveyance is not so frequent as is generally accepted. I have never from the first taken the position that it never occurs, but I still firmly believe that a tubercle bacillus which has passed from cow to cow for a long period, while it becomes more and more virulent for cattle, becomes less and less virulent for man, so that under ordinary conditions we have not so much to fear from milk and other products containing these bacilli, so far, that is, as the fully developed adult is concerned; but with weakly, young children the case is different. They are susceptible, and if a large dose of tubercle bacilli be given to them in the milk, I firmly believe that even a relatively slightly virulent bovine tubercle bacillus may gain entrance into their system in such large numbers that the cells are unable to kill them, and that here or there they may gain a point of growth, and once they grow they may gradually adapt themselves to the human organism and so set up the fatal disease. I doubt if this necessarily occurs in all children; we have, that is, cases brought forward in which children have been fed upon the milk of cows

[1] In view of the Interim Report of the British Royal Commission on Tuberculosis, I would lay special emphasis upon this point. That Commission has, in quite a number of cases, caused tuberculosis in cattle by the injection of human tubercle bacilli. Because disease can be transmitted experimentally by injection of a number of bacilli far in excess of the number which in nature could possibly gain entry at any one focus, it is by no means proved that under natural conditions these same bacilli are liable to cause infection. What the Commission should demonstrate, in order to establish that human tuberculosis is dangerous to cattle, is that the minimum dose of human tubercle bacilli capable of setting up tuberculosis in cattle approximates to the minimum dose of bovine bacilli producing the like effect. This I am convinced is not the case. There may be examples of bovine infection of man in which the bacilli still retain the high grade of virulence for cattle, but everything indicates that these are the exceptions.

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suffering from tuberculosis of the udder, without showing a sign of the disease.[1] Still, undoubtedly, the danger is there.

Here I would only say that certain very interesting observations recently published support my view that the relative frequency of tuberculosis of the intestine of children must not be ascribed positively to drinking the milk of tuberculous cows; it may equally well be due to swallowing saliva containing breathed-in tubercle bacilli, discharged into the air from the lungs of men and women suffering from the disease, or may have been sucked up by the fingers after a child has been crawling on the floor. And these are observations by one of the greatest bacteriologists of our time, the man who first gained pure cultures of the tetanus bacillus, and who was one of the first to work out the antitoxin treatment in infections, the discoverer also of the plague bacillus, and that man is the great Japanese bacteriologist, Kitasato.

Nowadays we have a thorough and wholesome respect for the Japanese and his methods, and not the least for his thorough knowledge and practical application of bacteriology. The nation which has taken to heart the dictum of King Edward, "If preventable, why not prevented?" has applied bacteriologic methods in the conduct of warfare, sending bacteriologists with each division, which has had the wisdom to recognize that *le Général Microbe* would be for her a far more powerful ally than Czar Nicholas' *Général Février*, that from the experience of recent wars for every one Russian put out of action by shot, or shell, or bayonet, four would be invalided by pestilence, and, accepting the warnings and advice of the bacteriologists, has managed to much more than reverse these figures in her own army; that nation and the bacteriologists of that nation deserve our respect. It is a recent paper by Kitasato that I wish to bring before you.[2] In this, with a wealth of statistical data, he has shown that the deaths from tuberculosis in Japan are just about in the same proportion to the total deaths and the total population as are the deaths from this disease in

[1] It must not be thought that I recommend that milk from tuberculous cattle should be drunk with impunity; there is something repugnant in the idea that milk containing any form of infective disease should be used as a food. The fullest precautions should be taken and legislation developed to prevent the use of milk from animals suffering from any form of infective disease.

[2] *American Medicine*, January 7, 1905

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European countries. There is, in fact, a remarkable similarity in his tables, suggesting very strongly that the factors at work are identical. In the second place, though here I confess his data might be fuller, he shows that in those under 18 years of age the number of cases of evident primary intestinal tuberculosis is certainly not less, but on the contrary, rather more than among Europeans and Americans, namely, 30% of the total deaths from this disease, whereas in Europe of late there has been a rather remarkable consensus of observations giving the proportion at about 25%. But, as I have already stated, it is usual to attribute these cases in early life to infection from cow's milk, while von Behring goes so far as to attribute most human tuberculosis to this cause—the use of cow's milk in infancy. But now, says Kitasato, the use of cow's milk for feeding infants is unknown in Japan; if a mother is unable to feed her child a foster mother is employed. Singularly little milk is consumed in Japan, and a careful calculation made from the total population, from a census of milch cows throughout Japan, and the average daily amount of milk yielded per cow, indicates that the individual Japanese on an average consumes daily just about three-quarters of a teaspoonful of cow's milk. Even in Tokio, the largest city, where most milk is consumed, the amount per individual works out to two and a third teaspoonfuls.

Next it is shown that bovine tuberculosis is unknown among the native Japanese cattle, while by cross-breeding with imported European cattle they become infected. Experimentally, subjected to a severer test than is ever likely to occur in nature, a certain number can be given the disease. A few, very few, cases have been reported in which the disease has been notified as found in native cattle, and this is only in Tokio and Yokohama where most foreign cattle have been introduced and these socalled native cattle may have been of mixed breed; for, in accordance with Mendel's law a certain proportion of cross-breeds are likely to have the characters of the native sire or dam, and be indistinguishable from the native race.

To epitomize: The facts gathered in Japan show that intestinal tuberculosis, which is as frequent there as in Europe, cannot be attributed to the ingestion of infected cow's milk, cannot therefore be of bovine origin, and the inevitable conclusion is that if intestinal tuberculosis is moderately frequent and not of bovine origin, then

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similarly, a large proportion of the cases of European intestinal tuberculosis are in all probability not due to infection from milk. In other words, these observations support the view that I have maintained for the last six years that undue stress is laid upon tuberculosis as a source of human infection. The danger is there; do not let me be misunderstood, I am convinced that weakly children are susceptible to the disease conveyed through the milk of cows suffering from udder tuberculosis; only the danger has been exaggerated. With Koch I hold that infection in the great majority of cases is from man to man, and that our main efforts should be in the direction of preventing such infection.

This does not mean that I would restrict the legislation regarding tuberculosis in cattle. Far from it. These observations of Kitasato support what I have urged all these years, that it is possible to eradicate bovine tuberculosis independently of our efforts to eradicate the disease in man. Kitasato points out that so far as the chronicles of Japan extend back through the centuries they tell of the existence of human tuberculosis, and yet, although the disease has been present all these centuries, the cattle of the present day are not infected. If the human strain of bacilli easily adapt themselves to existence in the bovine organism this could not be possible. This is another link in the chain of evidence which led me to urge in 1899 before the Canadian Medical Association,[1] and repeatedly since, that we in Canada should lead the world in completely banishing the disease from among our cattle. The disease is altogether too prevalent in European countries, for example, for this to be possible—the cost there would be too great. We are remarkably free from the disease, freer than any other western country, still it exists and its eradication is obviously a national and not a provincial concern. We are told that the Federal Government hesitates to interfere in the prevention of human tuberculosis, not because it does not recognize that this is a work of national import, but because doing this it would be trespassing upon provincial rights, and the law is above the people and their welfare. But the health of animals has been from confederation onward, a matter both of national concern and of federal legislation. I would once again urge that it is for the government to select some one well-defined section of our country and there to root out completely the

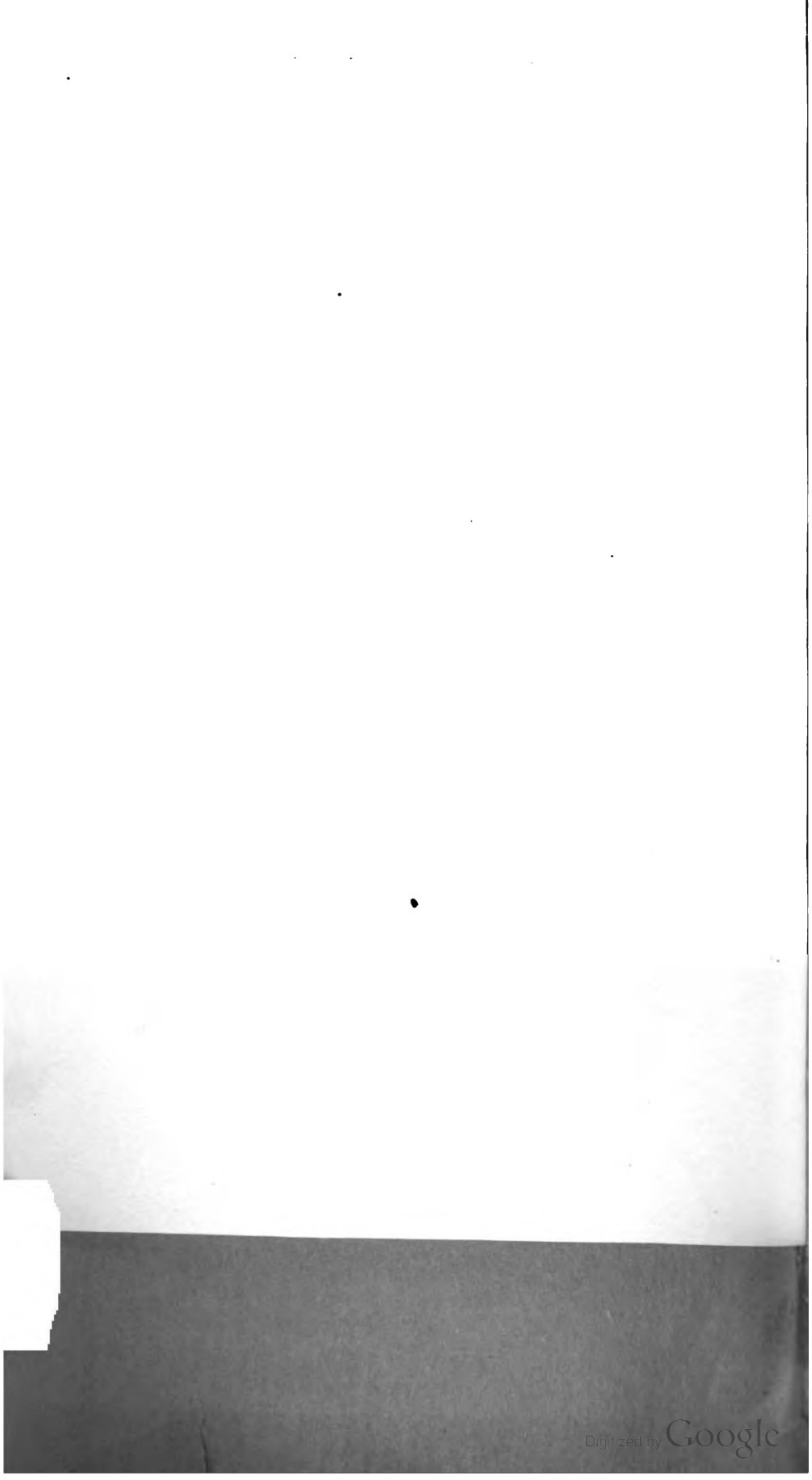
[1] Philadelphia Medical Journal, December 30, 1899

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disease from among the cattle. Let it take Prince Edward Island, for example, appoint inspectors, and be prepared to superintend the health of the animals on the Island for, say five years; let those inspectors make a census of all the cattle on the Island, let them apply the tuberculin test and take over and compensate for all reacting cattle; disinfect the byres and forbid any fresh animals to be landed without rigorous determination that those animals are free from the disease. I am convinced, first, that by the end of two years, if from the start they thoroughly enter into their work, the inspectors would find not a single case of the disease cropping up anew on the Island. That disease, I say, only passes from animal to animal and not from man to animal, under natural conditions, and if there are no infected animals to convey the disease, no new case can show itself. In the second place, the farmers would be benefited materially; no longer would they be subject to loss from the disease decimating their herds, and what is more, the certainty that their stock was free from the disease would enhance the value of that stock and afford a market for it for breeding purposes, not merely at home, but in distant countries which despair of obtaining uninfected animals, their own stock being so riddled with tuberculosis. And lastly, the experience gained in eradicating the disease in one locality would show how it can be accomplished most economically and surely in other parts, and eventually throughout the whole of the Dominion, so that Canada, our Canada, should stand before the world as the first country to solve the great problem and to possess stock wholly free from this devastating disease, so ruinous to agricultural communities throughout the world.

This is no chimeric plan; it is a perfectly feasible experiment, assured of success from the first, causing little disturbance and capable of being carried out at no great cost. If the Government has not merely the fear of the provincial politician before its eyes, but possesses a statesman-like foresight, if it has the welfare of the community at heart, the wellbeing of this Canada of ours, then I urge that it take up this work; that it make a forward move fraught with advantage to what is by far the greatest industrial interest of the country, even if it does not immediately see its way clear to take up measures for the benefit of the greatest national interest of all—the health and the well-being of the people.

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FROM THE

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VOL. XXXIX.

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ON THE LIMITS OF THE SENSIBILITY TO ODOURS AND OF THE METHODS OF DETECTING EMANATIONS.

By M. BERTHELOT.

(Contributed by Dr WALTER COLQUHOUN, Physiological Laboratory, University of Glasgow.)

IN the study of emanations, which has recently been so fruitful in results, the important question arises as to the order of magnitude of the quantities of matter involved. Is the order of magnitude comparable to that of the minute quantity of odoriferous substance in the air in contact with the olfactory mucous membrane which can be detected by odour? The writer published exact experiments on the limits of sensibility to odours in the *Annales de Chimie et de Physique*, 7e series, tome xxii. p. 460, 1901. By the exact methods described in that paper it was found that the limits of sensibility of the olfactory organ of the experimenter to the odour of iodoform, was the hundredth of one-billionth of a gramme disseminated in 1 cubic centimetre of air.

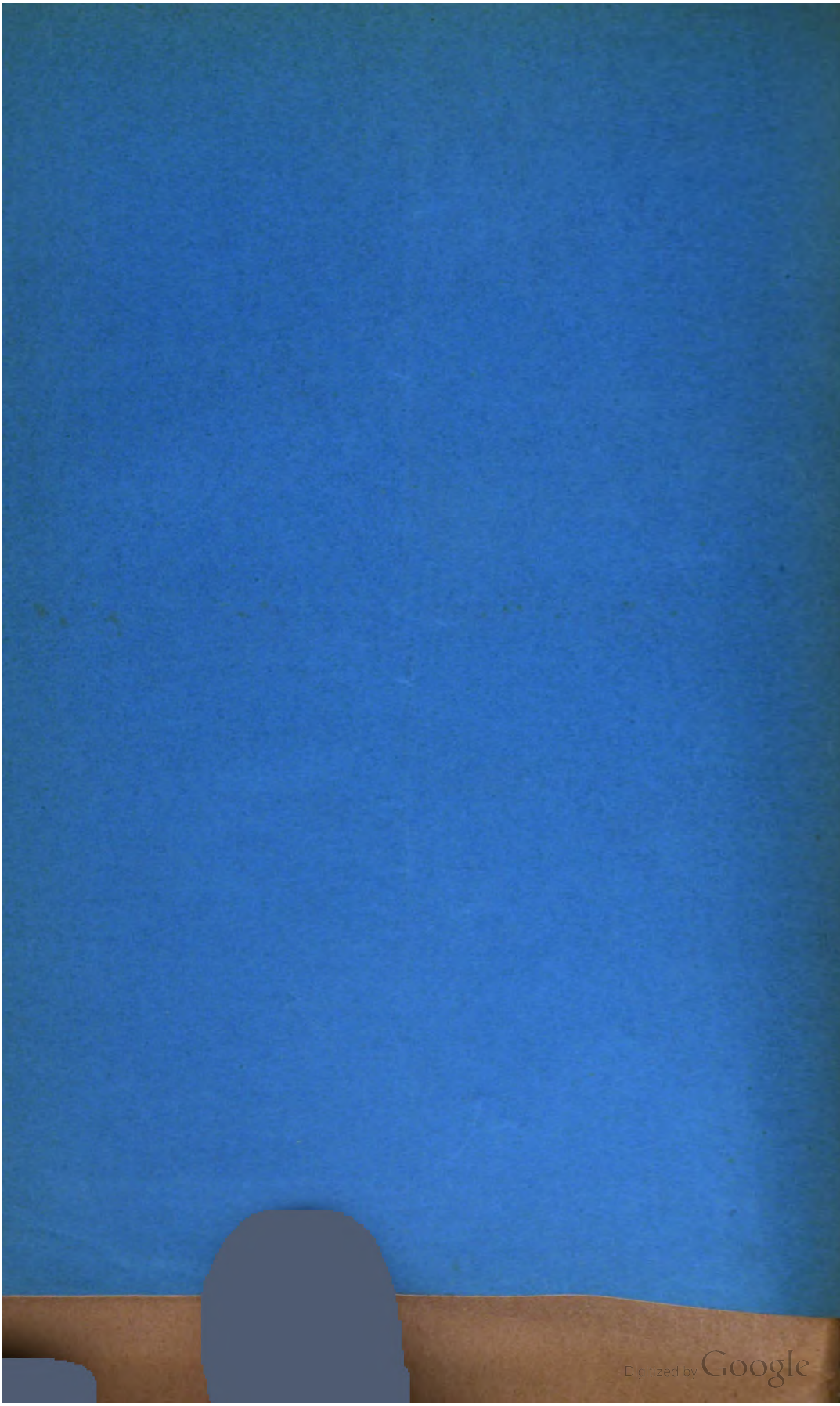
Suppose, now, a gramme of iodoform to be placed in a vessel of small capacity communicating by a tube, provided with stopcock, with a receiving vessel of 100 c.c. capacity, into which the volatilised matter enters. A cubic centimetre of air can be withdrawn at intervals from the 100 c.c. receiver to be tested by the nose for presence of odour. Suppose also that an hour elapses before the odour can be detected in the 1 c.c. of air withdrawn, and at the end of that time the receiver is removed and replaced by a similar one, and so on. Under the conditions stated, a gramme of iodoform would lose one-billionth of a gramme in an hour, and if the operations were continued for a year, it would lose $365 \times 24 = 8760$ billionths of a gramme, or rather less than one-hundredth of a milligramme. It would thus take one hundred years for the gramme of iodoform to lose one milligramme. Had musk been used instead of iodoform for the experiment, the weight lost would have been 1000 times less, according to estimations which have been made under less accurate conditions than those of the writer.

Such considerations prove that chemists, when using a delicate means of investigation such as the spectroscope, which in its sensitiveness may be compared to the olfactory organ, should be cautious in their statements regarding decomposition of chemical elements. Thus, if, in investigating the properties of an apparently pure mass of a certain element, the properties of another element are indicated by the delicate means of detection employed, that is no proof of decomposition of the first element. It might be impossible to extract the second element from the first if its mass were only one-thousandth of that of the principal element. Yet, as has been pointed out, apparatus exists which can detect one-hundredth of one-billionth of a gramme of iodoform in 1 c.c. of air, and perhaps one-thousandth of that mass in the case of musk.

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REPRINTED FROM THE PROCEEDINGS OF THE ASSOCIATION OF AMERICAN ANATOMISTS, 1900.

GIFT OF THE COMPILER
C. R. BARDEEN.

BORN'S METHOD OF RECONSTRUCTION BY MEANS OF WAX PLATES AS USED IN THE ANATOMICAL LABORATORY OF THE JOHNS HOPKINS UNIVERSITY.

By Charles Russell Bardeen,

Associate in Anatomy, Johns Hopkins University.

The wax-plate method of reconstruction (Plattenmodellen methode) described by Born in 1876[1] has proved of great value in the study of the morphology of embryos. The method has received its most extensive application in the hands of Born, of His and of various pupils of these investigators. In general, however, it may be said, that the value of this method as an aid to the microscopic study of form has not been sufficiently appreciated.

In part this lack of a more general application of the method has been due to certain technical difficulties which tend to make it cumbersome and time-consuming. Yet by no other method can so accurate an idea be obtained of the form of those structures which from their minuteness or complexity of relation cannot well be dissected out.

Considerable application of the method has recently been made by different persons in this institution and each worker has contributed something towards making the method more effective.

As originally described by Born several steps are essential for the successful application of his method. These may be tabulated as follows:

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[1] Morph. Jahrb. II; Arch. f. mikr. Anat., xxii, p. 584.

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A. Preliminary steps.

1. Obtaining a good picture of the embryo or object to be reconstructed.

2. Hardening, staining and sectioning the object.

3. Drawing magnified enlargements of the sections or such parts of them as it is desired to reconstruct.

4. Preparation of the wax plates.

5. Transference of the image to the surface of the wax and cutting out the wax plates.

B. Constructing the model.

1. Piling the wax plates.

2. Removing parts not essential to the reconstruction desired and rounding off of the parts reconstructed.

3. Strengthening and finishing the model.

I shall consider these steps in the order named.

A. Preliminary steps.

1. Before proceeding to section the object to be reconstructed it is important to obtain good pictures of its external form. With such a picture at hand it is much easier to pile up the wax plates which represent the sections through the object. This is especially true when the object is symmetrical, as in the reconstruction of embryos, profile views of which are invaluable in this work. If the picture be enlarged to the magnification of the model desired a valuable control is furnished. A series of parallel lines may then be drawn through the picture to represent the planes through which the knife has passed in sectioning the embryo, so that the position of every plate is indicated.

For general purposes photography is undoubtedly the most convenient method of recording the gross external features of the object. If the object be very small as, for instance, an early human embryo, the camera may be so placed that the image in the negative is enlarged from two to four diameters. It is found that the most convenient way of photographing embryos is to place the camera with the axis in a vertical direction and the lens pointing downwards. A stand for holding the camera in this position and raising or lowering it is easily constructed. Ordinary lead shot seems to be

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especially good for holding many small objects in the position in which it is desired to photograph them.

For detail in the distant as well as the proximal part of the object it is a great aid to make use of a stand capable of being raised without moving the object laterally. In this way, if the diaphragm be closed down so as to make the exposure a long one, the object may from time to time be brought slightly nearer to the lens of the camera, so that parts more distant are brought into sharp focus.

From the photographic plates thus obtained lantern slides are made or the negative itself is used to project the image at the required magnification upon a screen. Free-hand drawings are then traced on a paper upon which the image falls, or, if desired, bromide enlargements can be made. In this way accurate records can quickly be made of the external appearance of the object to be studied, yet no special talent for drawing is required. In the study of embryos the profile view is the most essential one, though others also prove of great value.

2. The only real essentials in the technique of obtaining serial sections of the object to be studied are that the series should be complete, the sections perfect and of uniform thickness. As pointed out by Born, the most convenient sections for this work are those from 20-40 microns in thickness. For sections of this thickness we have found alum cochineal to give uniformly the most satisfactory stain. It is important to know which side of the sections was uppermost during the cutting, so that in the subsequent reconstruction a true and not a mirror image of the object will be formed. For this reason it is well to make it a uniform practice to begin at the head when cutting transverse sections through an embryo, at the right side when cutting longitudinal vertical sections, and at the dorsal side when cutting horizontal sections and to label the sections in the order in which they have been cut.

3. For making drawings of the sections we have found that in general a projection apparatus is more convenient than a camera lucida unless the sections are small. Our projection apparatus is set up in a large dark room.

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The illumination is received from an arc electric light or from a heliostat. An ordinary microscopic stand with the tube in a horizontal direction is used when the sections are small and a high magnification is desired. Eye piece and draw tube are usually removed and the objective is used as the magnifying lens. In case of larger sections a projection lens similar to that used for lantern slides is utilized.

The image is projected upon a screen which runs on a track. The screen can be moved toward or away from the microscope by means of windlass situated near by. In this way any desired magnification can be quickly obtained by using an appropriate lens and bringing the screen into the proper position.

The screen which I devised for our dark room has attached a leaf which can be lowered so as to form a drawing table and a mirror that can be placed at an angle of 45° over the table. In this way the image is projected on a horizontal surface so that tracing it is easier than when it is upon a vertical surface. In using an ordinary mirror a double image is projected but that from the surface of the mercury is so much brighter than that from the surface of the glass that no difficulty is experienced in drawing accurate outlines.

Fig. 1 illustrates the apparatus here in use.

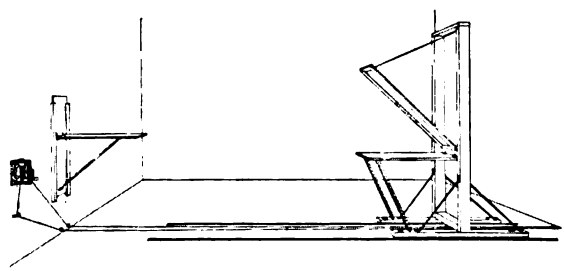


FIG. 1.—At the right the projection screen is shown in position on the track. The mirror is lowered to an angle of 45° and the drawing table is extended horizontally below this. At the left are shown the windlass used for moving the projection screen and the shelf used for holding the projection lantern.

In drawing pictures of the sections a careful outline of those main features which it is desired to bring out in the

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reconstruction is the great essential. In addition it is often of value to distinguish by using pencils of various colors the different organs in structures as they appear in the section.

If desired, direct bromide enlargements can be made of the sections on the slides. This is the method preferred by His. The simpler method described above we have found, however, to be more convenient for general purposes.

The outline drawings may often be elaborated to any desired extent when the sections are subjected to careful microscopic study. It is a great help for the subsequent reconstruction to label, so far as possible, the various structures in the outlines of the sections before proceeding to the wax plates.

4. Much trouble in the preparation of the wax plates is to be saved by using plates of a uniform thickness and by making the magnification of the object under reconstruction correspond. The most convenient thickness for general use is 2 mm. Occasionally, for coarser work, 4 mm. plates have proved of value. It is very easy, with the apparatus above described, to make the ratio of the diameter of magnification of the drawings to the diameter of the sections equal to that of two millimetres to the thickness of the section. If plates 2 mm. thick be used and every section be drawn, sections 20 mm. thick = 1/50 mm. must be magnified one hundred times. Or if desired, as is more often the case, every other section may be drawn at a magnification of fifty diameters.

For making the wax plates we have a large zinc pan with vertical sides. Its surface area is such that one kilogram of the wax mixture which we use will make a plate 1 mm. thick. The method of casting the plates is essentially that described by Born. Boiling water is run into the pan to the depth of several inches. On the surface of this the hot melted wax mixture is poured and quickly forms an even, smooth layer. Bubbles, which occasionally appear in the wax, may be quickly exploded by turning the flame of a Bunsen burner on the surface of the wax where they appear. As the wax plate cools it is necessary to free it from the sides of the pan by running a knife along the edge. Before the plates are perfectly cool they may readily be cut into smaller plates of any desired size.

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The wax mixture in use here is composed of 950 parts of bees-wax and 50 parts of white rosin. Often, especially in summer, paraffin is added to give additional toughness. Black plates are made by adding lamp black to the melted wax, until after thorough stirring the mixture has become uniformly black. The amount by weight of wax necessary for a plate of a given size is obtained more easily by experimental trial than by calculation. A certain amount of wax becomes attached to the sides of the pan by surface tension, so that slightly more wax must be used than the amount one is likely to determine by calculation from the specific gravity of the wax and the size of the pan. On the other hand if a pan of a given size be used the amount of a given wax mixture necessary for making a plate of given thickness may be determined by a few trial castings.

The outlines are transferred to wax by means of red or blue tracing paper. The wax plates are then placed upon glass and are cut with a small, narrow knife and in a warm room.

B. Constructing the model.

1. The janitor can be trusted to trace the outline drawings on wax, to cut through the wax with a sharp knife where the outlines are traced and to make the preliminary piling. Usually two preliminary piles are made, one of that part of the wax plates which represent the sections and one of the wax plates themselves after removal of the parts representing the sections. From the former a positive, from the latter a hollow negative image of the original object is obtained. In this piling an enlarged picture of the object is of very great help. As originally suggested by Born, in case of symmetrical objects a surface outline may be drawn on card board and cut out, thus giving a fixed ridge against which to pile the plates. If but one side of any embryo is to be reconstructed from transverse sections it is of great help to cut each plate off sharply at the midline and to pile the plates against a profile outline of the embryo situated on a board which has been placed perpendicular to the plane in which the plates are piled. In case the reconstruction of some

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internal organ is wanted it is usually of advantage to reconstruct at the same time the external form of the object, so that when the plates are piled the image they form may be compared with the picture of the original object. After getting the plates composing the positive image of the object into proper position, it is easy to trace two or three of its surface curves on paper or to represent them in wire and then to get the negative formed, as described above, into true shape. Plaster casts can then be made in this negative mould. The plaster casts, representing the external features of the original object, are very valuable to have at hand, while engaged in reconstructing the internal features from the wax plates.[2]

The method of making every fifth plate a black one has proved to be extremely valuable in arranging the wax plates. In this way it is easy at any time during the reconstruction of the model to count up and place any given section.

The method of reconstruction which I have found most convenient is as follows: After the plates are placed in proper position so that the external features of the object are accurately portrayed, I begin by taking off five plates from one side. The drawings of the sections I likewise have pinned together in groups of five in the same order in which the plates are piled. By going over the five finished drawings it is easy to obtain a good conception of the form of the structures represented in the block of five plates under consideration. I have at hand a paper of fine pins and these I press down through the various structures seen in section on the surface plate, and in such a direction that they will pass into the same structure in the sections below. When the parts of the plates which represent the structures to be reconstructed are thus firmly united by pins I remove the intervening portions of the wax plate with a pair of forceps. Thus, in a very short time, one is enabled to bring to light the form of the structures lying within the block of five sections. The pins hold the various bits of wax firmly in

[2] Many methods have been devised of piling plates according to special marks. The method devised by Wilson, Zeitschrift für wissenshaftliche Mikroscopie, xvii, 1900, page 177, seems a good one.

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place and serve to strengthen the model in every way. When I feel satisfied with the appearance of the structures in the first block of five sections I proceed to the next and treat it in the same way. Those structures which are cut in both blocks of sections may at the same time be pinned together. After two or three blocks of sections have thus been piled up it is often well before adding another block of five sections to fuse them together with a hot knife and thoroughly to strengthen the reconstruction so far as it is completed. For strengthening piles of narrow strips of wax, representing sections through membranes and the like, a wire netting is of the greatest value. Perhaps the best form of wire netting for general purposes is a copper netting with 10 strands to the centimetre. The copper netting has no tendency to cause subsequent warping, as is the case with iron netting. The netting is heated in the flame of a Bunsen burner and is then applied to the surface which it is desired to strengthen. In case of narrow columns, such, for instance, as are formed in the reconstruction of blood-vessels and nerves, copper wire is of the greatest value. This can be heated and sunk in at one side and then fused over.

After the model is once well started the subsequent building up can proceed with great rapidity. Plates in blocks of five are added as described above until the model is finished. Of course a greater or less number of plates than five may be used to a block. In most of my work, however, I have found blocks of five, with a black plate on the surface of each block, to give the most satisfactory results.

In order to keep the various structures distinct during the reconstruction it is often of value to paint them with different colors, while the work proceeds. The various structures of a model built up as described may be removed as completed, or during the course of reconstruction, and then readily replaced. Pins are of great value in holding structures in place and for indicating where a structure removed must be replaced in order to regain its proper position.

If it is desired at any time to cut the model in a given direction the pins which hold the pieces of wax together may be readily cut with scissors.

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3. I have mentioned methods by which the model is greatly strengthened during the course of reconstruction, the use of pins, of wire netting and of wire. All three means may be employed thoroughly to strengthen the model after the first rough reconstruction. The wire screening is then especially valuable. Of course it is possible to add free hand and with a good deal of accuracy structures which from their delicacy are difficult to model. This is true of blood-vessels, nerves and of fine membranes. The blood-vessels and nerves may be readily constructed by covering copper wire with wax, the membranes by covering a netting of narrow meshes with a thin coating of wax.

In rounding and smoothing up various structures in a model so as to give it a finished appearance, semi-melted wax applied with the fingers or with a spatula is of the greatest help.

The model is greatly protected in many ways by a thick coating of paint. Hot weather seems to have a far less detrimental effect on such models than on models unpainted.

We have found photography of great help not only in recording the condition of the finished model but also, at times, during the course of a reconstruction.

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Omaggio dell'a.

5

LABORAT. DI ISTOLOGIA E FISIOLOGIA GENER. DELLA R. UNIVERS. DI ROMA
DIRETTO DAL PROF. G. MAGINI

DOTT. PIERO CHIARINI

CAMBIAMENTI MORFOLOGICI

CHE SI VERIFICANO NELLA

RETINA DEI VERTEBRATI

PER AZIONE DELLA LUCE E DELL'OSCURITÀ

PARTE I

LA RETINA DEI PESCI E DEGLI ANFIBI

Estratto dal Bollettino della R. Accademia Medica di Roma

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Dott. PIERO CHIARINI. — Cambiamenti morfologici che si verificano nella retina dei vertebrati per azione della luce e dell'oscurità.

Parte I.

La retina dei pesci e degli anfibi.

Poche parole di introduzione saranno sufficienti per dichiarare lo scopo di questo lavoro, ed i metodi che ho seguìto nelle mie ricerche.

Mi sono proposto di rivedere le modificazioni morfologiche, che avvengono per azione della luce e dell'oscurità nella retina dei vertebrati, con l'intenzione non tanto di trovare fatti nuovi, sfuggiti agli osservatori che mi hanno preceduto in questo studio, quanto di ricercare quello che c'è di sicuramente dimostrato e dimostrabile in questo argomento.

Mi sono limitato a studiare, come è dichiarato anche nel titolo, i soli mutamenti morfologici, lasciando completamente da parte la questione della porpora retinica, e tutti gli altri cambiamenti chimici e microchimici, che sono stati osservati nella retina per azione della luce; ed ho sperimentato soltanto l'azione della luce bianca, senza occuparmi delle luci monocromatiche, nonchè di altri agenti fisici e chimici, che pure sono sufficienti a indurre cambiamenti morfologici negli elementi retinici, perchè se avessi troppo allargato il campo delle mie ricerche, mi sarebbe riuscito assai difficile di controllare tutto; ed era invece mia intenzione di non affermare, se non quello che ho potuto vedere da me personalmente.

In questa prima parte, che vede ora la luce, e che è destinata allo studio della retina dei pesci e degli anfibi, ho riferito soltanto i fatti anatomici, senza cercarne la spiegazione, e senza

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trarre delle conclusioni, che saranno meglio a posto nella seconda parte, dopo che avrò studiato anche i cambiamenti che avvengono nella retina dei rettili, degli uccelli e dei mammiferi.

Ho posto ogni cura perchè gli esperimenti riuscissero immuni da qualsiasi causa di errore; ed in generale, per avere il massimo della differenza, ho paragonato animali tenuti allo scuro per non meno di 24 ore, con animali che avevano subito per parecchie ore di seguito l'azione della luce solare diretta; ma non ho trascurato di studiare anche gli effetti della luce diffusa. Agli animali che rimanevano per parecchie ore esposti al sole, ho cambiato spesso l'acqua, per eliminare l'azione del riscaldamento.

Per lo studio sui pesci mi sono servito del *Leuciscus aula B.*, per lo studio sugli anfibi ho scelto la rana, controllando sempre i risultati con esperimenti analoghi fatti sul tritone.

Mi sono servito dei più svariati liquidi fissatori, ed ho ottenuto i risultati migliori col liquido del Flemming, e per le rane ed i tritoni anche con i vapori di acido osmico, dalla soluzione al 2 per cento nella camera umida; buono mi si è mostrato anche il liquido di von Tellyesniczky (bicromato di potassio al 3 % + acido acetico dal 3 al 5 %), che conserva ottimamente gli elementi istologici della retina. Risultati meno buoni ho avuto col sublimato, col sublimato acetico, e, per questi vertebrati inferiori, anche con l'acido nitrico e con la formalina.

Ho fatto quasi tutti i tagli servendomi della inclusione in paraffina; la inclusione in celloidina, che ho tentato più volte, con la speranza di veder meglio conservati i rapporti fra i vari elementi istologici della retina, mi ha sempre dato risultati meno buoni.

Per la colorazione, salvo casi speciali, ho adoperato la saffranina unita all'acido picrico, e la ematossilina di Ehrlich unita alla eosina.

Cenno storico.

Enrico Mueller, basandosi sul fatto già provato sperimentalmente dal Purkinje, che in certe determinate condizioni è possibile vedere i vasi della propria retina, dette per primo una

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dimostrazione convincente del fatto, ammesso già dal Treviranus fino dal 1835, che i coni ed i bastoncelli sono gli elementi della retina sensibili alla luce; ma rimaneva ancora completamente ignoto il modo col quale questi elementi reagiscono verso gli stimoli luminosi. Spetta al Boll il merito di avere per primo sollevato un lembo del velo, che avvolgeva da ogni parte questo oscuro problema, e di avere gettato le basi per una teoria più razionale della visione.

Nel suo memorabile lavoro " Sulla anatomia e fisiologia della retina „ (1) presentato all'Accademia dei Lincei nella seduta del 3 dicembre 1876, oltre alla scoperta della porpora retinica, che forma l'oggetto principale della memoria, egli parla anche del fatto importante, veduto da lui per primo, della migrazione del pigmento dell'epitelio retinico sotto l'azione della luce bianca e delle luci monocromatiche, intravvedendo tutta la importanza, che questo fatto aveva per la funzione visiva della retina, tanto che afferma essere sua convinzione " che le unità fisiologiche, le quali percepiscono la luce ed i colori, sono organismi anatomici assai complicati, e che essi debbono essere costituiti dalla riunione dei bastoncelli e dei coni da un lato, e delle cellule del pigmento retinico dall'altro „; e più oltre, parlando delle alterazioni da lui osservate nello strato a mosaico e nel pigmento della retina, dice che esse " formano la base materiale sulla quale, in avvenire, si costruirà la teoria fisiologica completa della visione e della percezione dei colori... „ " stabilendosi che, come negli organi dotati di nervi ad azione centrifuga (muscoli, organi elettrici e luminosi), così anche negli organi di senso, dotati di nervi ad azione centripeta, corrispondono agli stati fisiologici del riposo e dell'attività determinate alterazioni materiali di natura fisica, chimica ed anatomica „.

Le ricerche, così felicemente iniziate dal Boll, furono proseguite dal Kühne (5) che si dedicò specialmente allo studio della porpora retinica, e dall'Angelucci (17), il quale descrisse la contrazione delle cellule dell'epitelio pigmentato, e parlò anche di una contrazione dei bastoncelli. Ma gli sfuggì la contrazione del membro interno dei coni, la quale costituisce il fatto più saliente, che si verifica nello strato dei coni e dei bastoncelli, e che fu scoperta dal Van Genderen Stort nel 1884, completan-

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dosi così la serie dei più importanti cambiamenti anatomici, che si verificano nella retina sotto l'azione della luce.

Nell'anno successivo, 1885, Engelmann (9) mise in rilievo la diretta dipendenza dei movimenti dei coni e delle cellule pigmentate della retina dal sistema nervoso, dimostrando che basta esporre alla luce un solo occhio di una rana, per avere la discesa del pigmento e la contrazione dei coni anche nell'occhio rimasto all'oscuro; e che si possono ottenere gli stessi fatti nei due occhi, anche facendo agire la luce soltanto sul dorso e sulle estremità posteriori della rana, mentre gli occhi sono mantenuti nell'oscurità.

Queste osservazioni fondamentali sopra l'azione che la luce esercita sulla retina, furono poi confermate ed ampliate da una serie di ricercatori, e non è mancato anche chi le ha in parte contradette. Dei loro lavori avrò occasione di occuparmi in seguito, per ora basti avere accennato a grandi tratti la storia dell'argomento.

I.

Lo studio dell'azione della luce sopra la retina dei pesci è stato fatto recentemente dal Pergens (27) sul *Leuciscus rutilus L.* Io, come ho già accennato, ho istituito le mie ricerche sopra il *Leuciscus aula B.* Per comodità di studio, seguendo l'esempio del Pergens, ho mantenuto l'antica divisione della retina in dieci strati, che verranno passati successivamente in rassegna.

Epitelio pigmentato. L'epitelio retinico del *Leuciscus aula B.* è fatto da un solo strato di cellule, a contorni regolarmente poligonali, in genere con sei lati, le quali si presentano molto alte e strette. Vi si possono distinguere due parti: un corpo fortemente pigmentato, dove si trova il nucleo e poche goccioline grassose di luteina, ed i prolungamenti molto lunghi e robusti, che si insinuano fra i bastoncelli ed i coni. Il pigmento retinico, detto anche *fuscina*, si presenta per la massima parte in forma di bastoncelli, una piccola parte è in forma di granuli più o meno fini.

Il Pergens ha trovato che i due fenomeni più appariscenti, che si producono nell'epitelio pigmentato per azione della luce, sono anche nel Leuciscus la migrazione del pigmento verso la

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membrana limitante esterna (*), e la contrazione della cellula epiteliale. Egli ha trovato che " nelle retine di Leuciscus rimaste all'oscurità la massa pigmentaria forma una sola zona che occupa il limite esterno delle cellule, e si estende per un quarto circa della lunghezza delle cellule medesime, nelle retine esposte alla luce invece il pigmento è ripartito in due zone, fra le quali si scorgono dei tratti pigmentati, che occupano la parte mediana del protoplasma cellulare; le retine esposte alla luce solare diretta mostrano una rarefazione completa del pigmento nella porzione esterna delle cellule epiteliali, ed il pigmento è accumulato in una massa serrata in vicinanza dei coni e bastoncelli „. " Le cellule epiteliali che nell'oscurità misurano fino a 120 micromillimetri di lunghezza, alla luce ordinaria misurano in media 76 micromillimetri, e dopo cinque minuti di esposizione alla luce solare 72 micromillimetri „.

Il Pergens avrebbe osservato inoltre, che il nucleo il quale occupa nell'oscurità la parte più esterna del corpo cellulare vicino alla coroide, si muove per azione della luce, portandosi verso la metà della massa cellulare. Egli si è occupato anche di stabilire il grado di progressione del pigmento verso i coni, in relazione con la durata della eccitazione luminosa, ed ha trovato che " mentre nell'oscurità i prolungamenti della cellula epiteliale sono debolmente pigmentati, già dopo due minuti di esposizione alla luce contengono una maggior quantità di pigmento; dopo cinque minuti il pigmento, che si è fatto sempre meno abbondante nella porzione esterna della cellula, tende ad accu-

(*) A scanso di equivoci, credo utile dichiarare, che parlando della retina e dei suoi elementi, io mi servo delle parole *esterno* ed *interno*, per indicare con la prima la parte della retina rivolta verso la coroide, cioè verso la parte *esterna* o periferica del globo oculare, e con la seconda la parte che è rivolta verso il vitreo, cioè verso la parte *interna* o centrale del globo oculare. Ho creduto di dovere adottare questa nomenclatura, che non è la più esatta, perchè ormai i termini *esterno* ed *interno*, applicati alle membrane limitanti della retina e ai due strati granulosi, sono talmente radicati nell'uso, che volendoli cambiare si correrebbe rischio di generare confusione, e se si conservano per le membrane limitanti e per i granuli, non c'è ragione di non applicarli anche agli altri elementi della retina.

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mularsi nella regione vicina ai coni e bastoncelli, accentuandosi così la tendenza alla formazione di due zone pigmentate, separate da uno spazio chiaro. Prolungando l'azione della luce, anche se questa non è molto intensa, la zona pigmentaria esterna si assottiglia sempre di più, mentre si ispessisce proporzionatamente quella interna „. Mettendo nell'oscurità le retine che sono state esposte alla luce, il Pergens ha osservato i fenomeni inversi: " già dopo un minuto si nota un ritorno graduale del pigmento verso la parte esterna periferica delle cellule epiteliali ; dopo cinque minuti si vedono le due zone separate da uno spazio meno pigmentato, e dopo venti minuti il pigmento è accumulato in una sola zona, dalla quale si partono delle frangie colorate „.

Nelle retine di Leuciscus rimaste lungo tempo allo scuro (24-48 ore) io ho trovato il pigmento raccolto quasi completamente entro il corpo della cellula epiteliale, che acquista l'aspetto di un blocco oscuro, nel quale non è possibile vedere nè il nucleo nè le goccioline di luteina. Solamente all'estremo limite esterno, ad immediato contatto della lamina vitrea della coroide, si vede un sottilissimo alone di protoplasma sprovvisto quasi di pigmento. Nelle sezioni condotte perpendicolarmente alla superficie della retina, il pigmento si presenta sotto la forma di una fascia bruna, situata nella parte esterna della retina, a contatto della coroide, la quale occupa la quarta parte circa dell'altezza, che corre dal limite periferico delle cellule epiteliali fino alla membrana limitante esterna. Il bordo interno di questa fascia, rivolto verso la limitante esterna, va gradatamente sfumando, e termina sfrangiato con prolungamenti, che si insinuano per tratti molto brevi fra i bastoncelli ed i coni. (Vedi tavola fig. 1).

Nelle retine che sono state esposte alla luce chiara diffusa, o meglio alla luce solare diretta, il pigmento ha abbandonato quasi totalmente il corpo cellulare, per insinuarsi entro i prolungamenti e portarsi per la massima parte nella metà interna dello spazio, che corre fra la limitante esterna e l'estremo coroideale delle cellule epiteliali ; qui esso forma una fascia molto meno compatta di quella che si trova all'estremo limite coroideale delle retine rimaste nell'oscurità. Da questa fascia si partono robuste frangie sia in direzione della coroide, sia in di-

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rezione della membrana limitante esterna. Queste ultime frangie non oltrepassano di regola l'elissoide dei coni, e solo eccezionalmente qualche bastoncello di pigmento si spinge molto vicino alla limitante esterna senza arrivare mai a toccarla. (Vedi tavola fig. 2).

Nelle retine esposte alla luce diffusa non ho notato sempre la ripartizione del pigmento nelle due zone descritte dal Pergeus: quando è molto chiara e la si lascia agire per un tempo abbastanza lungo, anche la luce diffusa è sufficiente per provocare lo spostamento completo del pigmento, anche nei pesci che non hanno prima soggiornato all'oscuro. La disposizione del pigmento in due zone è invece molto evidente, nei pesci che sono stati esposti a una luce molto scarsa. Posso invece anche io confermare pienamente il fatto già osservato dal Kühne (5) sulle rane, che sotto l'azione della luce il pigmento retinico si consuma. Nelle retine che furono esposte lungamente alla luce solare diretta, ed anche alla luce diffusa molto chiara, la quantità del pigmento è evidentemente diminuita, e si vedono nel corpo cellulare e nella parte superiore dei suoi prolungamenti una quantità di granuli completamente scolorati, i quali verosimilmente sono granuli di fuscina, che hanno perduto il loro pigmento; tuttavia non si può affermare con sicurezza che siano veramente tali, perchè riesce difficile differenziarli dai granuli di altra natura, che si trovano nel protoplasma della cellula epiteliale.

La contrazione della cellula pigmentata sotto l'azione della luce appare evidente nel corpo cellulare, che si fa assai più basso (vedi tavola figg. 1 e 2), anche i prolungamenti della cellula subiscono senza dubbio un accorciamento, ma io debbo ritenere esagerate le cifre del Pergens, il quale avrebbe trovato una differenza di quasi 50 micromillimetri fra le cellule che sono rimaste all'oscurità, e quelle che hanno subìto l'azione diretta della luce solare, e di oltre 40 micromillimetri per quelle che sono rimaste alla luce diffusa. Il Pergens non dice come ha fatto le sue misure, e se è facile comprendere, che egli ha preso come uno dei limiti il margine libero coroideale delle cellule pigmentate, non si capisce quale è l'altro limite, che non può essere la membrana limitante esterna, perchè per

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la distanza che corre fra questa ed il margine coroideale delle cellule pigmentate egli dà altre misure più ampie, e se ha preso come limite il punto più basso a cui giunge il pigmento, le misure non possono avere alcun valore, perchè è un limite che varia fra troppo ampie oscillazioni. È perciò, che pur ritenendo come certo che la cellula epiteliale della retina del Leuciscus si contrae sotto l'azione della luce, prudenza vuole che non si esprima la cosa con cifre, le quali non possono avere un valore preciso.

Neanche ho potuto constatare il movimento del nucleo di cui il Pergens parla, perchè il forte accumulo di pigmento entro il corpo cellulare maschera completamente il nucleo, il quale è visibile solamente nelle cellule epiteliali delle retine che furono esposte alla luce, ed in queste esso non occupa una posizione costante entro il corpo cellulare.

Le mie ricerche collimano quasi con quelle del Pergens per quanto riguarda la progressione del pigmento verso la limimitante esterna, in relazione con la durata della eccitazione luminosa. Nelle retine tenute all'oscurità per 24 o 48 ore ho trovato il pigmento accumulato in totalità nel corpo delle cellule epiteliali, ed i prolungamenti poco o niente pigmentati, e sempre per tratti brevissimi. (Vedi tavola fig. 1). Sotto l'azione della luce solare diretta, già dopo un solo minuto incomincia ad accennarsi la migrazione del pigmento, che progredisce sempre gradatamente, muovendosi dal corpo cellulare verso i suoi prolungamenti. La migrazione del pigmento è continua e progressiva, ma non raggiunge il suo massimo se non dopo molto tempo. Occorre cioè qualche ora di azione continua della luce solare diretta, perchè il pigmento abbandoni completamente il corpo della cellula epiteliale, per portarsi tutto entro i prolungamenti. (Vedi tavola figg. 2-3-4-5).

Per ciò che si riferisce al movimento del pigmento in senso inverso, io ho notato che quando tutto il pigmento si è portato nei prolungamenti delle cellule epiteliali, per azione prolungata della luce solare diretta, occorrono per lo meno quattro o cinque minuti di permanenza della retina nell'oscurità, prima che si incominci a notare un evidente movimento del pigmento dai prolungamenti verso il corpo cellulare. Una volta che il movi-

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mento si è iniziato esso continua in modo progressivo, finchè quasi tutto il pigmento si è portato entro il corpo cellulare, per raggiungere il quale scopo occorre un tempo non inferiore ad un'ora. (Vedi tavola figg. 6-7-8).

Potrebbe sembrar strano il fatto, che mentre lo spostamento del pigmento dal corpo della cellula epiteliale verso i suoi prolungamenti si inizia più presto del movimento inverso dai prolungamenti verso il corpo cellulare, per raggiungere poi il massimo dello spostamento del pigmento occorre invece un tempo maggiore nel primo che nel secondo caso. Non è difficile però rendersi ragione di questo fatto se si pensa che per l'azione prolungata della luce solare diretta il pigmento si è in parte consumato, e che quindi essendo minore la quantità del pigmento, che si deve portare dai prolungamenti nel corpo della cellula epiteteliale, è naturale che questo spostamento si compia in un tempo più breve.

Studiando la migrazione del pigmento dai prolungamenti verso il corpo delle cellule epiteliali della retina del Leusciscus, ho potuto mettere in evidenza un altro fatto nuovo, che sta probabilmente in relazione con la diminuzione della quantità totale del pigmento nelle retine, che furono esposte per lungo tempo all'azione della luce solare diretta, ed è che il pigmento, il quale abbandona i prolungamenti, non va ad occupare tutto il corpo cellulare, ma lascia quasi completamente libera una zona protoplasmatica piuttosto ampia, situata all'estremo coroidale della cellula epiteliale. Questa zona incolora si mantiene quasi libera di pigmento, anche quando tutto il pigmento si è nuovamente portato dai prolungamenti entro il corpo cellulare (vedi tavola fig. 8); io la ho trovata nel modo più evidente anche nelle retine che rimasero nell'oscurità per tre ore; poi comincia gradatamente a diminuire, e dopo 15-20 ore è scomparsa completamente. Questa zona incolora manca completamente o quasi, nelle cellule epiteliali di retine che furono tenute nella oscurità, senza averle prima esposte alla luce solare diretta.

Non è facile dare una spiegazione pienamente plausibile di questo fatto, perchè non si capisce come i granuli ed i bastoncini di pigmento non riescano a penetrare nella zona più esterna del corpo delle cellule epiteliali, mentre prima, si trovavano in

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gran quantità; io penso tuttavia che si possa trovar la ragione di questo fatto, ammettendo che i bastoncini ed i granuli di fuscina, dopo aver consumato il loro pigmento per azione della luce solare, risalgano dalla parte più bassa dei prolungamenti verso il corpo cellulare per far posto a nuovi granuli e bastoncini forniti di pigmento. In questo modo, dopo una lunga esposione della retina all'azione della luce solare diretta, si accumulerebbe nel corpo delle cellule epiteliali una notevole quantità di granuli e di bastoncini di fuscina, che hanno perduto il loro pigmento. Quando poi la retina viene portata nell'oscurità i bastoncini ancor pigmentati, che dai prolungamenti migrano nel corpo delle cellule epiteliali, spingerebbero i bastoncini privi di pigmento nella porzione più esterna delle cellule epiteliali medesime, ad immediato contatto della coroide, dalla quale possono trarre il materiale necessario alla reintegrazione del loro pigmento.

Questa ipotesi, la quale ha molto del verosimile, e che sta in perfetta armonia con la funzione della fuscina, che è quella di reintegrare a sue spese le perdite subite dai bastoncelli e dai coni, quando hanno a lungo funzionato, potrebbe dirsi dimostrata qualora si potesse accertare, che realmente nella porzione più esterna del corpo delle cellule epiteliali si accumulano i bastoncini ed i granuli di fuscina privi di pigmento. Questo però non è facile, perchè, come ho già osservato, riesce quasi impossibile differenziare i granuli ed i bastoncini di fuscina scolorati dai granuli di altra natura, che si trovano in questa porzione delle cellule epiteliali.

Coni e bastoncelli. — I coni della retina del Leuciscus sono molto sviluppati, in confronto dei bastoncelli molto esili. Ve ne sono di varia grandezza, e la variazione oscilla fra limiti molto ampî, ma non è il caso di distinguerne quattro o cinque varietà, come fa il Pergens, basando la sua distinzione sopra criteri completamente errati. In tutti i coni si può distinguere un membro esterno assai piccolo che finisce a punta, ed un membro interno nel quale si distinguono nettamente un elissoide assai grande ed un membro mioide molto sviluppato, che si impianta a larga base sopra la membrana limitante esterna. Il nucleo dei coni è sempre situato nella base del membro mioide,

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subito al di sopra della membrana limitante esterna; lo strato granuloso esterno è quindi costituito unicamente dai nuclei dei bastoncelli (vedi tavola fig. 1). Questi, come già ho detto, sono molto esili e lunghi; il loro spessore infatti è di circa 3,5 micromillimetri, e la loro lunghezza è in media di 118 micromillimetri. Nella metà nasale della retina i bastoncelli sono alquanto più corti, e la loro lunghezza va rapidamente diminuendo nella porzione equatoriale dell'occhio verso l'*ora serrata*.

Nelle retine che sono state tenute all'oscurità, tutti i coni si presentano molto lunghi, con un membro mioide lungo e stretto (vedi tavola fig. 1); nelle retine, che hanno subìto l'azione della luce, i coni sono invece corti con un membro mioide corto e largo (vedi tavola fig. 2). Il membro mioide dei coni più grandi misura in media 68 micromillimetri nelle retine tenute allo scuro e 15 micromillimetri in quelle che hanno subìto l'azione della luce solare diretta. Gli elissoidi ed i membri esterni dei coni non presentano differenze degne di nota nelle retine tenute allo scuro ed alla luce; di modo che nel Leuciscus l'accorciamento dei coni è dovuto essenzialmente alla contrazione del membro mioide, come aveva già notato Van Genderen Stort (12), che fu il primo ad osservare anche nei pesci l'accorciamento dei coni sotto l'influenza della luce. Per produrre l'accorciamento dei coni non è necessaria la luce solare diretta; il medesimo effetto si ottiene con la luce diffusa, anche se è scarsa.

Il Pergens asserisce che l'accorciamento dei coni si fa bruscamente: " una retina la quale dopo esser rimasta all'oscurità, subisce l'azione della luce per un minuto, presenta ancora l'accumulo del pigmento nella parte esterna delle cellule epiteliali, mentre che i coni ed i bastoncelli sono già contratti; la contrazione dei coni sembra dunque — dice il Pergens — essere il primo e più immediato risultato prodotto dall'arrivo della luce al fondo oculare. Questa contrazione è brusca, mentre la migrazione del pigmento è progressiva „. Dalle mie esperienze risulta invece, che la contrazione dei coni non è così brusca come dice il Pergens. Anche sotto l'azione della luce solare diretta i coni, specialmente i più grandi, impiegano un tempo abbastanza notevole per giungere al massimo della contrazione,

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e la contrazione si fa gradatamente dal principio alla fine. Certamente la contrazione dei coni giunge al suo massimo molto prima che tutto il pigmento sia passato dal corpo della cellula epiteliale nei suoi prolungamenti, ma fra gli elissoidi dei coni ed il pigmento non esiste mai quel notevole distacco, che il Pergens descrive e disegna nella fig. 4 della sua tavola.

Il Pergens non dice come ha eseguito le sue esperienze, non dice cioè se dopo aver fatto agire la luce sopra gli occhi per uno, due, tre minuti, ecc., ed averli messi nel fissatore, li ha poi mantenuti alla luce o nell'oscurità. Io ho fatto due serie di esperienze: nella prima dopo aver fatto agire la luce solare diretta per uno, due, ecc., minuti sopra gli occhi di pesci che uscivano dall'oscurità, ho decapitato i pesci mettendone le teste nel liquido fissatore, e le ho poi chiuse in apposita cassetta e portate nella camera oscura, in modo che il fissatore spiegasse la sua azione nell'oscurità; nella seconda serie di esperienze ho lasciato le teste dei pesci decapitati alla luce solare diretta. Nell'un caso e nell'altro il risultato è stato identico, ed ha dimostrato che la contrazione dei coni non si fa in modo brusco, come asserisce il Pergens, ma per gradi, procedendo quasi di pari passo con la migrazione del pigmento (vedi tav. figg. 3, 4, 5).

Nè più vero è il fatto, affermato dal Pergens, che " gli occhi di pesci, i quali vengono messi nell'oscurità, dopo aver subito l'azione della luce viva, subiscono prima l'allungamento dei coni e poi la migrazione del pigmento „. Anche in questo caso il movimento dei coni si fa gradatamente, e va di pari passo con la migrazione del pigmento; chè se fosse vero quanto asserisce il Pergens, gli elissoidi dei coni, dopo uno o due minuti di soggiorno dell'occhio nell'oscurità, dovrebbero trovarsi fra la coroide ed il pigmento retinico rimasto ancora vicino alla membrana limitante esterna, o quanto meno dovrebbero rimanere completamente coperti dal pigmento retinico, il che non è (vedi tav. figg. 6, 7, 8).

Lo studio dei bastoncelli nel Leuciscus riesce alquanto più difficile di quello dei coni, per la loro esilità e per la difficoltà che hanno ad assumere le sostanze coloranti. Tuttavia son riuscito a metterli bene in evidenza, in stato di perfetta conservazione, sia nei preparati fissati con liquido di Flemming, in

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alcuni dei quali ho potuto anche tingerli con la safranina, sia nei preparati ottenuti per dissociazione dagli occhi fissati con i vapori di acido osmico e macerati in alcool al terzo. Lo studio accurato di questi preparati mi ha persuaso che non esistono differenze degne di nota fra i bastoncelli delle retine tenute allo scuro e quelli delle retine tenute alla luce. Nell'un caso e nell'altro i bastoncelli presentano uno spessore di 3,5 micromillimetri e una lunghezza media di 118 micromillimetri. Non posso dunque convenire col Pergens, quando parla di contrazione dei bastoncelli; e francamente non riesco a capire come egli abbia potuto parlarne, perchè quando dovrebbe trattare dei bastoncelli, confessa di non averli potuti studiare per la loro esilità.

Strato granuloso esterno. — Lo strato granuloso esterno è costituito soltanto dai nuclei dei bastoncelli, i nuclei dei coni, come già abbiamo veduto, stanno tutti al di sopra della membrana limitante esterna, entro il peduncolo o membro mioide dei coni stessi.

Il Pergens avrebbe osservato che nelle retine di Leuciscus tenute allo scuro i nuclei dello strato granuloso esterno sono allungati, si colorano in bleu con la miscela di Biondi-Heidenhain, e sono molto ricchi di cromatina; nelle retine invece, che sono rimaste per un certo tempo sotto l'azione della luce, i granuli esterni sono tondeggianti, si colorano più debolmente in bleu con la miscela di Biondi-Heidenhain, e sono notevolmente più poveri di cromatina. Egli ha veduto infatti, che nell'oscurità i granuli esterni misurano $2,5 \times 7$, 3×10 e 3×6 micromillimetri, e alla luce 5×5, 3×5 e raramente $3,5 \times 7$ micromillimetri; e desume la diminuzione della cromatina nei granuli esterni delle retine tenute alla luce dal fatto, che incollando le une al lato delle altre sopra un medesimo vetrino sezioni di retine tenute alla luce e di retine tenute all'oscurità, e trattandole per dieci minuti con la miscela di Biondi-Heidenhain, dopo un rapido lavaggio nell'acqua distillata, ha veduto che lo strato granuloso esterno si è colorato in bleu nelle sezioni di retine rimaste all'oscuro ed in rosso in quelle di retine tenute alla luce: risultati analoghi dice di aver ottenuto trattando le sezioni con altre sostanze coloranti nucleari (fucsina,

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violetto di metile, bleu di metilene, dahlia, ecc.), le quali tutte coloravano più intensamente le retine rimaste nell'oscurità. Un altra conferma di questo fatto l'avrebbe trovata nell'osservazione, che la diminuzione della cromatina è specialmente manifesta nelle porzioni centrali della retina, le quali sono più esposte all'azione della luce.

Le mie ricerche, anche per questa parte, non sono in accordo completo con quelle del Pergens. Per quanto riguarda la modificazione della forma dei granuli esterni, ho trovato anch'io, nei preparati fissati con sublimato acetico e con acido nitrico, un po' più allungati i granuli delle retine tenute all'oscuro, che quelli delle retine tenute alla luce; ma non credo di dover dare molta importanza a questa osservazione, perchè nei preparati fissati con liquido di Flemming, i quali hanno conservato meglio i rapporti e la forma dei varî elementi istologici, non ho più trovato questa differenza. Dalle mie misure risulta infatti che tanto nelle retine tenute alla luce quanto in quelle tenute allo scuro sono più frequenti le dimensioni 3×7, più rare le dimensioni 4×6, rarissime le dimensioni 2×8 micromillimetri. In conclusione dunque mi pare di poter affermare che non ci sono modificazioni degne di nota nella forma dei granuli esterni delle retine tenute alla luce, in confronto con quelli delle retine tenute nell'oscurità. Nè più fondata mi è parsa la pretesa diminuzione della cromatina dei granuli esterni sotto l'influenza della luce. Io ho ripetute le esperienze del Pergens attaccando sopra un medesimo vetrino sezioni di retine tenute allo scuro e di retine tenute alla luce, e ho avuto cura di accoppiare sempre sezioni di un identico spessore, e precisamente di 10 micromillimetri; ho adoperato tagli di retine fissate in liquidi diversi, e li ho colorati con i colori più svariati, non esclusa s'intende la miscela di Biondi-Heidenhain, che preparavo al momento di servirmene, ma non ho potuto ottenere i medesimi risultati del Pergens. Adoperando i colori di anilina (verde di metile, bleu di metilene, rosso magenta, fucsina, violetto di genziana, ecc.) io ho avuto dei risultati contradittorî, perchè mentre qualche volta si coloravano di più le sezioni di retine tenute allo scuro, con non minore frequenza mi si sono colorate più intensamente le sezioni di retine tenute

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al sole, e non sempre tutte le sezioni di retine tenute allo scuro o al sole mi si sono colorate con eguale intensità; sul medesimo vetrino accanto ad una sezione molto tinta non raramente ne ho trovata una poco tinta, mentre ambedue provenivano dal medesimo occhio. Nè ciò può recar meraviglia a chi sa quanto riescano in genere irregolari e poco uniformi le colorazioni fatte con i colori di anilina, sicchè, se la sezione è un poco estesa, accanto a porzioni più tinte se ne vedono spesso altre meno tinte.

Neanche mi è riuscito vero alla prova il fatto affermato dal Pergens, che spingendo molto la decolorazione, si scolorano prima le retine sottoposte all'azione della luce, le quali sono già completamente scolorate, quando le retine tenute nell'oscurità mantengono ancora una evidente colorazione dei granuli esterni. Se invece dei colori di anilina, così infidi, si adopera il carminio o la ematossilina, si vede che i granuli di cromatina sono egualmente abbondanti ed intensamente colorati tanto nelle retine, che furono esposte all'azione della luce solare, quanto in quelle rimaste lungamente nell'oscurità.

Il Pergens evidentemente si è ispirato in queste sue ricerche ad un lavoro del Mann (24), il quale ha trovato che nelle cellule nervose la cromatina si accumula durante il riposo, e diminuisce durante il lavoro. Che il Pergens abbia avuto un' idea preconcetta, in queste sue ricerche sulla quantità di cromatina nelle retine tenute all'oscuro ed alla luce, risulta chiaramente dal fatto, che egli ha trovato diminuita la cromatina delle retine tenute alla luce anche in quei punti, che di cromatina non ne contengono affatto, come gli elissoidi dei coni.

Concludendo dunque io debbo dire, che lo studio accurato di preparati ben fatti dimostra, che nel Leuciscus non esistono differenze degne di nota nello strato dei granuli esterni di retine tenute nell'oscurità, in confronto con quello di retine, che furono sottoposte all'azione prolungata della luce solare diffusa o diretta.

Strato intergranuloso. — Oltre il plesso formato dalle espansioni protoplasmatiche e dalle arborizzazioni terminali dei piedi dei coni e dei bastoncelli e delle cellule bipolari, questo strato contiene nella retina del Leuciscus degli elementi cellulari volu-

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minosi, disposti in tre strati sovrapposti, situati in vicinanza dei granuli interni, elementi che sono stati descritti da Ramon y Cajal con il nome di cellule orizzontali, perchè mandano i loro prolungamenti in direzione orizzontale. Con i comuni metodi di colorazione si vedono i nuclei di queste cellule grandi ellittici, con il diametro maggiore in senso orizzontale e poveri di cromatina (vedi tav., figg. 1-2.)

Il Pergens ha osservato, che nelle retine esposte alla luce questi nuclei si fanno più tondeggianti, diminuiscono di volume e perdono un po' di cromatina.

Io non ho trovato differenze apprezzabili in questi nuclei nelle retine tenute alla luce ed all'oscurità. Quanto alla forma e alla grandezza ho veduto in prevalenza le misure 8 × 4, più rare le misure 10 × 4, rarissime le misure 6 × 4 micromillimetri, tanto nelle retine tenute all'oscuro che in quelle tenute alla luce, e nell'un caso e nell'altro ho veduto i nuclei piuttosto poveri di cromatina, ma non più poveri nelle retine tenute alla luce, che in quelle tenute allo scuro.

Strato granuloso interno. — Questo strato è fatto essenzialmente dalle cellule bipolari dei coni e dei bastoncelli, ma vi si trovano anche gli spongioblasti o cellule amakrine del Ramon y Cajal ed i nuclei delle fibre raggiate del Mueller.

Il Pergens descrive anche nei nuclei delle cellule bipolari un impiccolimento e una diminuzione notevole della cromatina per azione della luce. Io non posso confermare nè l'una cosa nè l'altra, perchè le ricerche più accurate non mi hanno fatto rilevare in questi nuclei differenze degne di nota fra le retine tenute all'oscuro e quelle tenute alla luce.

Strato molecolare. — In questo strato, che, come ha mostrato Ramon y Cajal, è fatto dall' intreccio delle arborizzazioni protoplasmatiche delle cellule gangliari, delle terminazioni delle cellule bipolari, delle ramificazioni terminali delle cellule amakrine, e delle appendici collaterali delle fibre del Mueller, il Pergens non ha osservato alcun cambiamento sotto l' influenza della luce; ed io non posso che confermare quanto il dice Pergens.

Strato delle cellule gangliari. — Le cellule nervose gangliari della retina del Leuciscus sono provviste di un grosso

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nucleo, povero di cromatina e circondato in genere da una quantità piuttosto scarsa di protoplasma, che contiene poca sostanza cromofila in forma di granulazioni; solo in rari casi, nelle cellule più grandi, la sostanza cromofila assume la forma di zolle.

Il Pergens dice, che nelle retine sottoposte all'azione della luce i nuclei delle cellule gangliari sono meno voluminosi e più poveri di cromatina; il protoplasma è retratto, meno abbondante, meno voluminoso, ed i prolungamenti protoplasmatici più spessi. Come conseguenza della retrazione del corpo cellulare si avrebbe un apparente ingrandimento degli spazî, nei quali sono contenute le cellule; ma in realtà secondo il Pergens questi spazî sarebbero invece rimpiccioliti per azione della luce.

Io ho studiato queste cellule su preparati fissati e colorati nei modi più svariati. Le immagini più belle si vedono nei preparati fissati con sublimato acetico, e colorati col bleu di metilene di Nissl o con la thioninà, ma la forma e la grandezza delle cellule appaiono meglio conservate nei preparati fissati con liquido di Flemming. Nei miei preparati non ho potuto osservare il rimpiccolimento del nucleo e la diminuzione della sua cromatina, nelle retine tenute alla luce; mi è parso invece di vedere un certo impiccolimento del corpo protoplasmatico, e una diminuzione dei granuli cromofoli del protoplasma; ma non credo di dover dare soverchia importanza a questi fatti, perchè, per la scarsità del protoplasma cellulare, e per la grande varietà della forma e della grandezza delle cellule medesime, i confronti fra le retine tenute alla luce ed allo scuro sono molto difficili, e dimostrano in ogni modo che, anche se esistono, le differenze debbono essere molto lievi.

Quanto agli spazî che circondano le cellule, io posso dire soltanto che nei preparati ben conservati questi spazî sono piccolissimi, perchè le cellule gangliarí li riempiono quasi totalmente; e ciò tanto nelle retine tenute allo scuro, quanto in quelle tenute alla luce. Debbo per ciò ritenere che il loro supposto ingrandimento sia dovuto al raggrinzamento del corpo cellulare per opera specialmente dei liquidi fissatori.

Nello *strato delle fibre del nervo ottico* non ho potuto trovare, per l'azione della luce, quell' ispessimento, del quale parla il

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Pergens, per quanto abbia messo ogni attenzione per osservarlo nei miei preparati.

In conclusione dunque mi pare si possa ritenere, che i soli cambiamenti morfologici veramente degni di nota, che si producono nella retina del Leuciscus per azione della luce solare diretta o diffusa, sono quelli che si riscontrano nell'epitelio pigmentato e nello strato dei coni e dei bastoncelli; e cioè: la contrazione della cellula epiteliale, la migrazione e la diminuzione del pigmento retinico, e la contrazione del membro mioide dei coni.

Nelle conclusioni del suo lavoro il Pergens insiste anche sopra un altro fatto, e cioè che nell'oscurità la retina è più spessa che alla luce. L'ispessimento sarebbe dovuto, secondo il Pergens, principalmente alla estensione dello strato dei coni e dei bastoncelli, che raggiungono la loro maggiore lunghezza nell'oscurità. Se alla luce la retina si fa meno spessa, ciò è dovuto, sempre secondo il Pergens, specialmente alla contrazione dei coni; gli altri strati della retina vi prenderebbero poca parte, come si può vedere dalle seguenti sue cifre:

	Oscurità	*Luce*
distanza fra l'estremità esterna dell'epitelio e la membrana limitante esterna.	126 μ	76 μ
dalla membrana limitante esterna fino a tutto lo strato granuloso esterno	17 μ	14 μ
dallo strato granuloso esterno fino a tutto lo strato intergranuloso	19 μ	16 μ
dallo strato intergranuloso fino a tutto lo strato granuloso interno	20 μ	17 μ
dallo strato granuloso interno fino alla limitante interna	42 μ	42 μ

In complesso si avrebbe dunque, che mentre la retina tenuta allo scuro ci presenta uno spessore di 224 micromillimetri, quella tenuta alla luce ha invece uno spessore di 165 micromillimetri; con una differenza di circa 60 micromillimetri in meno, dei quali 50 sono a carico dello spazio compreso fra l'estremità esterna dell'epitelio pigmentato e la membrana limitante esterna.

Per controllare questi dati del Pergens, io ho eseguito una gran quantità di misure cercando di eliminare, entro i limiti del

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possibile, le cause di errore, le quali sono molte e di non poca importanza, tantochè facilmente si può esser condotti a conclusioni in gran parte errate. Il Pergens dice che le cause di errore possono essere lo spostamento totale della retina rapporto alla coroide, e la obliquità dei tagli; ma altre e non meno gravi derivano dal fatto, che spesso gli elementi molto delicati della retina del Leuciscus, e specialmente gli esilissimi bastoncelli si piegano, e subiscono perciò una notevole diminuzione della loro lunghezza, diminuzione la quale tanto più facilmente può sfuggire alla osservazione, in quanto che è assai difficile di vedere i bastoncelli, specialmente nelle retine, che hanno subìto l'azione della luce. Ed anche può essere causa di gravi errori l'altro fatto, che le due metà, nasale e temporale, della retina non hanno un eguale spessore: la metà temporale è sempre molto più spessa della metà nasale; bisogna perciò star bene in guardia, quando si vogliono paragonare due retine, a scegliere dei punti che siano veramente corrispondenti. Finalmente debbo fare un'altra osservazione; ed è che anche nei tagli meglio riusciti, ed io ho posto ogni cura per ottenerne dei buoni, accade quasi costantemente di vedere delle differenze molto rilevanti nello spessore di zone limitrofe della retina.

Tuttociò dimostra quanto sia arduo stabilire con cifre lo spessore della retina e dei suoi varî strati, a proposito dei quali è bene notare, che tutti quelli situati al disotto della limitante esterna, se se ne accettua forse lo strato esterno dei granuli, non hanno limiti netti, sicchè riesce anche più difficile il poterli misurare esattamente. Per tutte queste ragioni mi sembra che non si possa dare molta importanza alle cifre, quando si tratta di stabilire e di paragonare lo spessore di due retine e dei loro strati, ed io tengo a dichiarare, che non credo affatto alla esattezza matematica delle mie cifre; pur tuttavia ho ragione di ritenerle molto più prossime al vero di quelle del Pergens. Per evitare, entro i limiti del possibile, le cause d'errore, ho proceduto nel modo seguente.

Ho paragonato, s'intende, soltanto le retine dei pesci di egual grandezza e trattate in modo identico, servendomi quasi esclusivamente di occhi fissati in liquido di Flemming, perchè questo fissatore mi ha dato i migliori risultati, quanto a con-

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servazione degli elementi e dei loro reciproci rapporti ; ho avuto cura di scegliere per le mie misure soltanto quelle sezioni, che presentavano l' ingresso del nervo ottico, ed ho eseguito sempre dieci misure, sia nella metà nasale che nella temporale, prendendole a distanze regolari di 25 centesimi di millimetro a partire dall' ingresso del nervo ottico per procedere verso l'equatore del globo oculare ; ho poi fatte le medie di una gran quantità di misure eseguite su retine tenute per parecchie ore alla luce solare diretta, e su retine tenute allo scuro per 24 ore ; ed ho ottenuto così delle cifre che rappresentano la media dello spessore della retina del Leuciscus, nella sua porzione più spessa attorno all' ingresso del nervo ottico, per un raggio di circa due millimetri e mezzo. Non ho compreso nelle misure la parte equatoriale della retina, perchè qui il suo spessore va rapidamente assottigliandosi. Ciò premesso ecco la tabella delle mie misure in micromillimetri :

	Oscurità		*Luce*	
	metà temporale	*metà nasale*	*metà temporale*	*metà nasale*
Epitelio pigmentato e strato dei coni e bastoncelli fino alla limitante esterna . . .	150	140	130	120
Strato granuloso esterno.	16	16	16	16
Strato intergranuloso	22	21	22	21
Strato granuloso interno.	21	20	21	20
Strato molecolare	30	29	30	29
Cellule gangliari e fibre del nervo ottico fino alla limitante interna.	24	20	24	20
Spessore totale della retina . . .	263	246	243	226

Come si vede, confrontando la mia tabella con quella del Pergens, tutte le mie cifre sono alquanto superiori a quelle del Pergens, ma questa divergenza può esser dovuta alla differenza di varietà e di grandezza dei pesci che abbiamo adoperato, e forse anche alla differenza del fissatore, sicchè non può avere

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grande importanza. Le divergenze importanti fra le mie e le cifre del Pergens si notano invece, quando si confrontano i rapporti fra le misure delle retine tenute all'oscuro e quelle delle retine tenute alla luce. Infatti il Pergens ha trovato una differenza di 50 o 60 micromillimetri fra le retine tenute allo scuro e le retine tenute alla luce, secondo le mie cifre invece questa differenza è soltanto di 20 micromillimetri; e la differenza è tutta a carico di quella porzione di retina, che è compresa fra la limitante esterna e la lamina vitrea della coroide, tutti gli altri strati della retina mantengono uno spessore eguale alla luce e allo scuro. Io credo che la grave divergenza fra le mie e le cifre del Pergens sia dovuta al fatto che il Pergens non ha tenuto conto di due cause di errore, e cioè di non aver paragonato fra loro porzioni di retine veramente corrispondenti, trascurando le differenze che esistono fra le due metà temporale e nasale della retina del Leuciscus, e di aver trascurato completamente i bastoncelli, i quali molto facilmente si presentano piegati e tortuosi. Questa circostanza ha una grandissima importanza, perchè lo spessore dello strato dei bastoncelli e dei coni dipende unicamente dai bastoncelli, ed il Pergens cade in errore quando afferma che " se alla luce la retina diventa meno spessa, è sopra tutto alla contrazione dei coni, che questo risultato deve essere attribuito „. Infatti tanto nelle retine tenute alla luce (vedi tavola fig. 2) quanto in quelle tenute allo scuro (vedi tavola fig. 1) i coni sono sempre notevolmente più corti dei bastoncelli, e non possono perciò avere alcuna influenza sullo spessore della retina. L'aver tenuto conto solamente dei coni, ci fa comprendere come il Pergens abbia potuto trovare naturale una differenza così enorme fra lo spessore delle retine tenute allo scuro e alla luce, perchè i coni subiscono veramente per effetto della luce una notevolissima contrazione; ma questa non può portare una diminuzione dello spessore della retina, perchè i bastoncelli, che sono assai più lunghi, e che non si contraggono, impediscono che questo effetto si produca. Che ciò avvenga lo dimostrano ad evidenza quei preparati di retine tenute lungamente sotto l'azione della luce solare diretta, nei quali (vedi tavola fig. 2) si vedono i bastoncelli continuarsi al di sopra dello strato del

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pigmento, ed urtare contro il corpo delle cellule epiteliali, che si son fatte notevolmente più basse.

Se è vero dunque il fatto, che nel Leuciscus sotto l'influenza della luce si ha una diminuzione dello spessore della retina, non è men vero altresì, che questa diminuzione è molto più leggiera di quanto afferma il Pergens, ed è dovuta quasi esclusivamente alla contrazione delle cellule epiteliali pigmentate, perchè i bastoncelli non si contraggono, e la contrazione dei coni non può avere nessuna influenza sullo spessore della retina.

II.

Lo studio dei cambiamenti morfologici, che si producono nella retina degli anfibi per azione della luce e dell'oscurità, è stato fatto da una gran quantità di osservatori. Infatti a cominciare dal Boll, che fu il primo ad iniziare questo studio, quasi tutti si sono serviti delle rane, le quali si prestano per queste ricerche meglio di altri animali, perchè la loro retina reagisce in modo assai manifesto agli stimoli luminosi, e perchè è sempre molto facile il procurarsele. Per queste ragioni, e anche perchè il mio lavoro non poteva essere se non un controllo, di ciò che era stato fatto dai precedenti osservatori, ho creduto bene di scegliere anch' io la *Rana esculenta,* come soggetto delle mie ricerche sugli anfibi, avendo cura tuttavia di controllarle sempre con ricerche analoghe eseguite sul tritone, (*Triton tentatus*).

Epitelio pigmentato. — L'epitelio pigmentato della rana è costituito, come quello del Leuciscus, da un solo strato di cellule, che vedute di faccia presentano una figura regolarmente poligonale, quasi sempre con sei lati; osservate di profilo presentano invece un aspetto cilindrico, e vi si possono distinguere con l'Angelucci (4) tre porzioni: la cupola, la base e i prolungamenti. La cupola si presenta generalmente priva di pigmento, e contiene il nucleo provvisto di uno o due nucleoli; accanto al nucleo si trovano delle goccioline oleose, costituite da una speciale sostanza detta *luteina* dal Capranica per il suo aspetto giallo dorato; nella cupola epiteliale si trovano anche dei granuli rotondi o cubici, scoperti per la prima volta dal Boll (1),

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che li ritenne erroneamente di natura grassosa, e li chiamò *granuli aleuronoidi*. Ewald e Kühne (5) li differenziarono dal grasso, e li dissero granuli mieloidi, perchè rammentano per alcune loro reazioni la mielina dei nervi. La base della cellula epiteliale, che contiene abitualmente la maggior parte del pigmento, presenta sulla sua faccia interna, rivolta verso la limitante esterna, una gran quantità di sottili prolungamenti protoplasmatici, i quali si spingono a guisa di ciglia fra i bastoncelli e i coni. Il pigmento, che si presenta per la massima parte sotto forma di bastoncelli non molto lunghi ad estremità piuttosto puntute, non ha una localizzazione costante entro la cellula epiteliale.

Come Boll (1) ha veduto per primo, il pigmento, che nelle rane tenute allo scuro si raccoglie quasi tutto nella base della cellula epiteliale, migra sotto l'azione della luce, e si porta nei prolungamenti protoplasmatici, insinuandosi fra i bastoncelli ed i coni, fino a raggiungere la membrana limitante esterna, al di là della quale non è stato mai osservato in nessun caso neanche un granulo di pigmento. La migrazione del pigmento, entro le frangie dell'epitelio per azione della luce, è accompagnata da un maggior grado di coesione fra l'epitelio medesimo e lo strato dei coni e bastoncelli; tanto che mentre nelle rane rimaste lungamente nell'oscurità è sempre facile separare i rimanenti strati della retina dall'epitelio pigmentato, nelle rane che furono esposte alla luce, la separazione non riesce così netta, e la retina ordinariamente si straccia in parecchi brandelli, ai quali si trovano attaccate porzioni più o meno grandi di epitelio retinico. Il Boll osservò anche, che mentre le retine esposte alla luce rossa si comportavano come quelle rimaste nell'oscurità, le luci verde, azzurra e violacea avevano un'azione quasi identica a quella della luce bianca sulla retina della rana.

La migrazione del pigmento non è il solo fatto, che si osserva nell'epitelio retinico della rana per azione della luce; come Boll (1) aveva già veduto, e Kühne (5) ha poi meglio stabilito, le goccioline oleose giallo dorate di luteina si fanno più chiare, e si scolorano anche completamente sotto l'azione delle luce; è allora assai difficile differenziare le più piccole di queste dai granuli mieloidi, e ci si riesce soltanto con l'aiuto

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di speciali reagenti (etere, cloroformio, acido osmico, ecc.). Angelucci (6) ha dimostrato inoltre che mentre nelle retine di rane tenute allo scuro la cellula epiteliale, veduta di profilo, si presenta alta, con la cupola protoplasmatica e la base pigmentata bene sviluppate, nelle retine di rane esposte alla luce la cellula epiteliale è bassa, con la cupola e la base assottigliate. Si ha insomma una vera e propria contrazione della cellula epiteliale sotto l'azione dello stimolo luminoso.

Il Fick (18, 20, 21) ha messo in dubbio che la migrazione del pigmento retinico della rana dalla base del corpo cellulare entro i suoi prolungamenti, sia dovuta all'azione della luce. Egli avrebbe trovato infatti, che nelle rane tenute al buio per una settimana il reperto più frequente è la discesa del pigmento fino alla membrana limitante esterna, oppure una speciale disposizione a spazzola del pigmento; e che se si espone una rana per cinque minuti alla luce, dando così la spinta alla migrazione del pigmento, esso continua a muoversi verso la limitante esterna anche nel buio; e che inoltre quando le rane sono state lungamente al buio, anche la luce diffusa è sufficiente a provocare la massima discesa del pigmento; e finalmente che nelle diverse porzioni della retina si trovano sempre grandi differenze nella disposizione del pigmento.

A queste obiezioni del Fick ha risposto fra gli altri il Gaglio (25), il quale, ripetendo ed ampliando gli esperimenti del Fick, è giunto alle conclusioni: 1° che la posizione interna del pigmento delle rane tenuta all'oscuro per una settimana, non è dovuta, come vuole il Fick, all'azione prolungata del buio, bensì all'edema marantico, al quale le rane soggiacciono, quando dimorano lungamente nella stessa acqua; infatti se si ha cura di cambiare l'acqua alle rane, il pigmento si trova sempre in posizione esterna, raccolto nella base della cellula epiteliale, anche quando le rane sono state tenute al buio per un tempo lunghissimo; 2°) che le differenze nella disposizione del pigmento sono notevoli soltanto nelle rane, che sono state esposte per breve tempo alla luce, ed in queste la discesa del pigmento verso la limitante esterna è sempre più accentuata nella parte centrale della retina, che meglio subì l'azione dello stimolo luminoso, e va degradando verso le parti equatoriali, le quali

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furono meno intensamente colpite dalla luce; 3°) che la durata del tempo impiegato dal pigmento a risalire alla posizione esterna aumenta col crescere della intensità luminosa dell'ambiente dal quale la rana proviene, e che viceversa la discesa del pigmento alla posizione interna è più rapida se la rana venne esposta alla luce diretta tratta dal buio che dalla luce diffusa.

Io convengo pienamente col Gaglio, quando combatte le idee del Fick, che sono in massima parte basate sopra esperimenti evidentemente mal riuscisti; e ne convengo perchè i miei preparati di retine di rane tenute all'oscuro e alla luce (vedi tavola figg. 9-10) dimostrano chiaramente tutti i fatti, descritti dal Boll dal Kühne e dall'Angelucci; ma non posso convenire col Gaglio quando asserisce (25), che se si espone una rana per mezz'ora alla luce solare diretta, si trova il pigmento in completa posizione interna, cioè disceso fino alla limitante esterna, in tutti i punti della retina, anche dopo due ore di esposizione al buio; perchè ho trovato, che dopo l'azione anche prolungata della luce solare diretta, la permanenza nell'oscurità per la durata di un'ora e mezza è sufficiente per far risalire completamente il pigmento dai prolungamenti entro la base delle cellule epiteliali della retina di una rana. Sarebbe stato infatti assai strano che, mentre nel Leuciscus già pochi minuti di permanenza nel buio sono sufficienti per dare al pigmento la spinta a tornare nel corpo delle cellule epiteliali, nella rana invece, che presenta il fenomeno della migrazione del pigmento quasi altrettanto accentuato quanto il Leuciscus, neanche due ore siano sufficienti per ottenere il medesimo effetto.

Strato dei coni e bastoncelli. — I coni della rana sono più piccoli e delicati dei bastoncelli. Se ne distinguono due varietà: gli uni, e sono di gran lunga i più comuni, posseggono una gocciolina oleosa situata al limite fra l'elissoide ed il membro esterno, gli altri ne sono privi. I primi sono mobili, si contraggono cioè sotto l'azione della luce, e si allungano nell'oscurità, i secondi rimangono sempre fissi, con l'elissoide molto vicino alla membrana limitante esterna. Anche dei bastoncelli, che sono molto più grandi e robusti dei coni (vedi tavola figg. 9-10), se ne distinguono due varietà: i più comuni, detti bastoncelli rossi, hanno un membro interno corto e largo, gli altri molto più rari,

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detti dal Boll bastoncelli verdi, hanno un membro interno lungo e sottile, ed un membro esterno molto più corto. I nuclei dei coni e dei bastoncelli giacciono tutti al disotto della membrana limitante esterna.

Sotto l'azione della luce i coni della rana si contraggono. Nelle rane che sono state tenute allo scuro (vedi tavola fig. 10) il membro mioide dei coni è lungo e sottile, tantochè l'elissoide ed il membro esterno si trovano spinti verso la coroide; l'elissoide si presenta allungato e piuttosto sottile, per trovar posto nello spazio limitato dai voluminosi membri esterni dei bastoncelli, fra i quali si è insinuato. Nelle rane invece che hanno subìto l'azione della luce (vedi tavola fig. 9) il membro mioide dei coni è cortissimo e largo, l'elissoide è situato molto vicino alla limitante esterna, e si trova compreso non più fra i membri esterni, ma fra i membri interni dei bastoncelli; contemporaneamente si è fatto più corto e più grosso, tanto che preme sul membro interno dei bastoncelli vicini. Secondo Van Genderen Stort ed Engelmann (9) si contrae solamente quella parte del cono, che ha conservato proprietà ottiche e chimiche, che rammentano quelle del protoplasma; e precisamente quella parte che va dalla limitante esterna fino al membro esterno del cono, rimanendo inattiva quella parte, che contiene l'elissoide del cono, come pure il membro esterno del cono.

Secondo Angelucci invece (17) prendono parte all'accorciamento anche l'elissoide del cono ed il suo membro esterno, che s'ingrossa e si contrae esso pure. Io ho trovato che tutto il membro interno dei coni, compreso l'elissoide, si fa più corto nelle retine, che sono state esposte all'azione della luce (vedi tavola fig. 9), ma non ho potuto notare nessuna differenza fra i membri esterni dei coni di retine di rane tenute allo scuro e alla luce, e penso anche, che abbiano ragione Van Genderen Stort ed Engelmann, quando affermano che la parte mioide del cono è la sola attiva, perchè credo che l'accorciamento e l'ingrossamento dell'elissoide, si possa meglio spiegare con la sua elasticità. Quando, per azione dell'oscurità, l'elissoide del cono viene spinto fra i membri esterni dei bastoncelli, che costituiscono come una fitta palizzata, deve subire un allungamento ed un assottigliamento, per potersi insinuare

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nello spazio ristretto compreso fra i membri esterni di quattro bastoncelli contigui; quando poi per azione della luce il membro mioide del cono si contrae, l'elissoide attratto in vicinanza della membrana limitante esterna, e liberato dalle strettoie dei membri esterni dei bastoncelli, può riacquistare la sua forma più globosa, perchè i membri interni dei bastoncelli, che sono costituiti da protoplasma non così altamente differenziato e di natura cuticolare, come quello dei membri esterni, non oppongono resistenza all'elissoide del cono. Che così sia veramente, mi conforta a crederlo anche il fatto, che gli elissoidi dei coni della retina del Leuciscus, i quali non trovano alcuna resistenza da parte dei sottilissimi membri esterni dei bastoncelli, conservano la medesima forma sia alla luce, sia allo scuro. Se non è esatto dunque, che nella rana l'allungamento e l'accorciamento dei coni, per azione dell'oscurità e della luce, siano dovuti soltanto a cambiamenti, che si verificano nel membro mioide, perchè anche l'elissoide cambia di forma, non è però men vero, che la parte, che si muove attivamente, è soltanto quella, che conserva proprietà ottiche e chimiche, simili a quelle del protoplasma.

Kühne (5), avendo osservato che nelle retine di rane tenute alla luce, i membri esterni dei bastoncelli, veduti di faccia, presentano le loro sezioni ottiche molto ravvicinate le une alle altre, mentre sono separate da uno spazio maggiore nelle retine tenute nell'oscurità, ritenne verosimile, che per effetto della luce si dovesse verificare un gonfiamento del membro esterno dei bastoncelli; ed a sostegno di questo suo modo di vedere, cita alcune misure di V. Hornbostel, il quale avrebbe trovato che nelle rane tenute allo scuro lo spessore dei bastoncelli oscilla fra 6 e 7 micromillimetri, e gli spazî interposti fra i bastoncelli misurano da 5 a 8 decimi di micromillimetro; mentre nelle rane tenute alla luce i bastoncelli stanno a mutuo contatto, e misurano da 6,8 a 7,2 micromillimetri.

Più tardi Angelucci (6) descrisse una vera e propria contrazione del membro esterno dei bastoncelli della rana per azione della luce, e quasi contemporaneamente al Gradenigo (10) parlò anche della contrazione del membro interno dei bastoncelli. Egli dice, che nelle rane tenuta allo scuro i bastoncelli

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sono esili, allungati tanto nel membro esterno che nell'interno, mentre nelle rane tenute alla luce i bastoncelli sono larghi e contratti tanto nel loro membro esterno che nella porzione mioide. L'Angelucci (17) insiste molto sulla contrazione dei bastoncelli, alla quale assegna un posto importante nei movimenti sincroni, che avvengono, come egli dice, per azione della luce sulla triade retinica, cioè sull'epitelio pigmentato, sui bastoncelli e sui coni.

Per questa ragione, ed anche perchè tutti gli scolari dell'Angelucci, che si sono occupati di questo argomento, hanno recisamente confermato la contrazione dei bastoncelli della rana, io ho messo ogni cura possibile nel ricercarla, ma dopo lunghe e pazienti ricerche, dopo ripetute esperienze, e molteplici misure, mi son dovuto persuadere, che non si può affatto parlare di contrazione dei bastoncelli della rana per azione della luce.

I bastoncelli della rana non hanno tutti il medesimo spessore e la medesima lunghezza. I più lunghi e sottili si trovano in corrispondenza del polo posteriore dell'occhio, attorno all'ingresso del nervo ottico; i più corti e più grossi si trovano nella parte equatoriale dell'occhio. Io ho trovato, che in corrispondenza del polo posteriore dell'occhio i membri esterni dei bastoncelli hanno in media uno spessore di 6 micromillimetri ed una lunghezza di 50 micromillimetri; nella porzione equatoriale, invece, i membri esterni dei bastoncelli hanno uno spessore medio di 8 micromillimetri ed una lunghezza di 30 micromillimetri. Come si vede le differenze sono molto notevoli, e bisogna star bene attenti, quando si vogliono paragonare i bastoncelli di due retine diverse, di sceglierli in porzioni di retine corrispondenti, per non cadere in errore. Seguendo appunto questo metodo, io ho potuto constatare nel modo più assoluto, che non esistono differenze fra i membri esterni dei bastoncelli di retine di rane tenute alla luce ed allo scuro. Neanche dopo l'azione prolungata della luce solare diretta, io ho potuto vedere l'accorciamento e l'ingrossamento dei membri esterni dei bastoncelli. Sicchè credo di poter affermare con certezza, che i membri esterni dei bastoncelli della rana non subiscono modificazioni di forma per azione della luce, cioè non si contraggono. Del resto si troverà razionale che sia così, se si pensa che, come

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ha dimostrato il Kühne (5), i membri esterni dei bastoncelli e dei coni sono formazioni cuticolari altamente differenziate, di composizione molto affine in parte alla sostanza cornea (neurocheratina) ed in parte alla mielina (sostanza mieloide), e che perciò non possono aver conservato la contrattilità del protoplasma non differenziato.

Nè può costituire una seria obiezione il fatto osservato dal Kühne (5) e dal V. Hornbostel, che nelle retine di rane tenute allo scuro, gli spazî interposti fra i membri esterni dei bastoncelli sono più ampî, che nelle retine di rane tenute alla luce; perchè questo fatto trova una spiegazione molto più razionale se si pensa, che appunto nelle retine rimaste allo scuro, gli elissoidi dei coni si trovano insinuati fra i membri esterni dei bastoncelli, i quali naturalmente debbono perciò venire alquanto allontanati gli uni dagli altri. Nelle retine che furono sottoposte all'azione della luce, invece, gli elissoidi dei coni sono attratti vicino alla limitante esterna, ed i membri esterni dei bastoncelli possono ravvicinarsi fra di loro.

I membri interni dei bastoncelli della rana sono molto più corti dei membri esterni e misurano in media 15 o 16 micromillimetri nei bastoncelli più lunghi. Quanto al loro modo di comportarsi nelle retine tenute all'oscuro e alla luce, io ho osservato quanto segue. Nelle retine di rane, che rimasero lungamente nell'oscurità (vedi tav. fig. 10) i membri interni dei bastoncelli si presentano ampî e larghi, di uno spessore eguale a quello dei membri esterni, senza presentare alcun rientramento ed assottigliamento nella parte mediana. Il bastoncello delle rane tenute allo scuro si presenta perciò con un calibro uniforme in tutta la sua lunghezza. Nelle retine di rane che sono state esposte alla luce (vedi tav. fig. 9) il membro interno dei bastoncelli presenta invece nella sua parte mediana un rientramento, un infossamento, nel quale si annida l'elissoide del cono, che si è fatto più globoso, ed è stato tirato vicino alla limitante esterna per la contrazione del membro mioide del cono. Questo rientramento della parte mediana del membro interno del bastoncello, che è dovuto certamente alla pressione esercitata dall'elissoide dei coni, e sul quale nessuno degli autori, che ho potuto consultare, aveva finora richiamata l'atten-

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zione, è la sola differenza, che ho riscontrato fra i membri interni dei bastoncelli di rane tenute alla luce, in confronto di quelli di rane tenute allo scuro. Io non ho potuto vedere nei membri interni dei bastoncelli l'accorciamento e l' ingrossamento per azione della luce descritti dal Gradenigo (10) e dall'Angelucci (17); che anzi, per la pressione che subiscono da parte degli elissoidi dei coni contratti, i membri interni dei bastoncelli sono piuttosto assottigliati nelle retine, che hanno subìto l'azione della luce. Dopo ciò mi sembra di poter affermare, che i bastoncelli della rana non subiscono alcuna contrazione per azione della luce.

Del resto l'accorciamento dei bastoncelli è stato messo in dubbio recentemente anche dal Greef (30), il quale dice, che le differenze nello spessore e nella lunghezza dei bastoncelli di retine tenute allo scuro e alla luce debbono essere molto insignificanti, e non sono dimostrate sicuramente, perchè piccole differenze esistono già fra i bastoncelli di un medesimo occhio, ed anche maggiori se ne trovano fra quelli di due occhi differenti.

Nel 1885 Engelmann (9), sperimentando sulle rane, osservò alcuni fatti, che dimostrano la diretta dipendenza dei movimenti dei coni e delle cellule pigmentate della retina dal sistema nervoso. Illuminando un solo occhio di una rana, constatò la migrazione del pigmento e la contrazione dei coni anche nell'altro occhio, rimasto nella oscurità ; la sola differenza nei due occhi, consisteva nel fatto, che i membri esterni dei bastoncelli dell'occhio illuminato erano completamente scolorati, mentre quelli dell'occhio tenuto allo scuro, erano intensamente colorati dalla porpora retinica. Questo fatto si osservava anchè nelle rane dissanguate o decapitate, purchè il cervello fosse conservato. Dopo la distruzione del cervello, l'azione della luce restava limitata al solo occhio illuminato. Per spiegare questa azione riflessa sui coni e le cellule pigmentate dell'altro occhio, Engelmann ammise che nei nervi ottici si debbono trovare anche delle fibre a conduzione centrifuga, che egli chiamò fibre retino motrici, perchè funzionano come nervi motori per i coni e le cellule pigmentate della retina.

Queste esperienze dell'Engelmann sono state contradette dal

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Fick (23), il quale ha negato anche l'esistenza delle fibre centrifughe del nervo ottico. Ma nonostante l'opposizione del Fick, le conclusioni dell'Engelmann hanno poi trovato piena conferma da altri osservatori, e specialmente da Lodato e Pirrone (32), ed anche prima dal Nahmmacher (22), il quale, per dimostrare la esistenza delle fibre retinomotrici, ha sperimentato sulle rane, stimolando sia l'occhio direttamente, sia il chiasma od il nervo ottico, non con la luce, ma col cloruro di sodio; ed ha trovato che nelle rane tenute allo scuro, quando la via dei nervi ottici è integra, si ha la migrazione del pigmento verso la membrana limitante esterna e la contrazione dei coni anche nell'occhio non stimolato. La reazione consensuale manca invece, quando uno dei nervi ottici è tagliato.

Del resto le fibre a conduzione centrifuga del nervo ottico sono state dimostrate dal Monakow con osservazioni anatomo-patologiche, dal Martin con ricerche embriologiche, e finalmente in modo diretto e indiscutibile dal Ramon y Cajal e dal Dogiel.

Io ho voluto ripetere l'esperimento dell'Engelmann, e per mettermi al coperto da ogni obiezione ho proceduto nel modo seguente. Ho preso due rane di egual grandezza, ho chiuso ad ambedue l'occhio sinistro, ravvicinando le palpebre con tre punti di sutura, ed applicando sulle palpebre chiuse un dischetto di carta bibula impregnato e coperto completamente con bitume di giudea; le ho poi messe allo scuro, e dopo trascorse 24 ore le ho ravvolte in un pezzetto di panno nero, e ne ho portata una alla luce solare diretta, procurando che rimanesse illuminato soltanto l'occhio destro, che era rimasto aperto. Dopo una mezz'ora circa di azione della luce solare diretta, ho decapitato la rana fissandone la testa con gli occhi in liquido di von Tellyesniczky, e subito dopo ho fatto altrettanto per la rana che era rimasta al buio. Ho avuto così gli occhi di due rane, che avevano subito l'identico trattamento, con la sola differenza, che l'occhio destro di una di esse era stato esposto per mezz'ora all'azione diretta della luce solare. Lo studio di questi occhi mi ha dimostrato, che mentre nella rana, la quale rimase sempre allo scuro, si trovavano i coni allungati ed il pigmento raccolto entro la base della cellula epiteliale, rimanendone quasi sprovvisti i prolungamenti; nel-

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l'altra rana invece il pigmento era migrato entro i prolungamenti delle cellule epiteliali, fin presso la limitante esterna ed i coni erano contratti; e ciò tanto nell'occhio destro, che fu esposto alla luce solare, quanto nel sinistro, che rimase sempre allo scuro.

In questo modo ho potuto evitare anche l'obiezione, che la migrazione del pigmento e la contrazione dei coni nei due occhi fosse dovuta al trattamento subito dalla rana, anzichè alla illuminazione di un solo occhio, perchè la rana di controllo, mantenuta nel buio e trattata nel modo identico, non ha presentato i medesimi fatti. Questa precauzione non parrà superflua se si pensa, che non soltanto la luce ma anche altri stimoli fisici e chimici sono sufficienti per provocare la migrazione del pigmento e la contrazione dei coni. Così, per citare qualche esempio, già Kühne (5) aveva veduto, e Gradenico (10) ha poi meglio dimostrato, che il calore agisce in modo analogo alla luce sopra le retine di rane mantenute nell'oscurità. Engelmann (9) ha dimostrato che il tetano stricnico ed elettrico producono la migrazione del pigmento e la contrazione dei coni nelle rane tenute al buio, ed Angelucci (17) ha trovato che in modo analogo agiscono anche il suono e l'edema marantico, al quale soggiacciono le rane, se si lasciano a lungo senza ricambiare l'acqua nella quale vivono.

Strato granuloso esterno. — I granuli esterni della retina della rana sono disposti su due piani, nel piano più esterno, subito al di sotto della membrana limitante esterna, si trovano i granuli dei bastoncelli, nel piano più interno i granuli dei coni (vedi tavola figg. 9-10).

Czerny (2) fu il primo ad osservare nella retina di rane, le quali gli servivano per certi suoi esperimenti sull'acciecamento mediante la luce solare, che gli elementi dello strato dei granuli esterni, nei punti dove la luce aveva agito direttamente sopra la retina, sono oblunghi, mentre si presentano tondeggianti nelle altre parti; ed attribuì la forma ovale dei granuli a fatti patologici. Il Gradenigo (10), studiando più tardi l'azione della luce e del calore sulla retina della rana, giunse a dimostrare, che questo allungamento dei granuli esterni si accompagna alle altre modificazioni morfologiche, che la retina

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subisce per azione della luce nell'epitelio pigmentato e nello strato dei coni e bastoncelli, e vide altresì che l'allungamento dei granuli esterni si ottiene non soltanto con gli stimoli luminosi, ma anche per mezzo del calore sulle rane mantenute allo scuro.

Ne concluse perciò ragionevolmente, che questa modificazione di forma dei granuli esterni non può essere un fenomeno patologico, ma deve piuttosto stare in rapporto con fatti funzionali. Le osservazioni del Gradenigo furono poi confermate anche dall'Angelucci (17) ed io stesso ho potuto constatare un evidente allungamento dei granuli esterni nelle retine di rane, che hanno subito l'azione della luce. Infatti i granuli esterni che nelle retine di rane rimaste allo scuro sono tondeggianti, e misurano in media 9 × 9 micromillimetri, nelle retine tenute alla luce misurano il più spesso 6 × 12 micromillimetri. L'allungamento mi è parso in generale più accentuato sui granuli dei bastoncelli, ma è sempre evidente anche nei granuli dei coni.

Qualche volta l'allungamento dei granuli è così accentuato, che essi hanno acquistato un aspetto fusiforme; ma io credo che questo fatto sia dovuto a difetti di preparazione, perchè non l'ho mai veduto nei preparati meglio riusciti, e perchè è quasi sempre molto più manifesto nelle parti equatoriali della retina, che sono state colpite meno intensamente dallo stimolo luminoso.

Denissenko (11 ed Angelucci (17) hanno descritto nelle retine, le quali furono esposte alla luce, anche un ingrandimento degli spazî, dove sono contenuti i granuli esterni; ingrandimento che io non ho potuto constatare nei miei preparati.

Per quanto riguarda gli *altri strati della retina* della rana Denissenko (11) dice di aver osservato, che per azione della luce gli spazî dove sono contenuti i granuli interni si fanno più ampî, i nuclei dei granuli interni divengono fusiformi, e s' ingrandiscono anche gli spazî dove sono contenute le cellule gangliari. Contemporaneamente le fibre del nervo ottico si separano le une dalle altre e si ispessiscono. Tutti questi cambiamenti condurrebbero ad un aumento dello spessore della retina sotto l'azione della luce.

Anche Angelucci (17) ha descritto l' ingrandimento degli spazi

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entro i quali sono contenute le cellule gangliari, nelle retine di rane che sono state esposte all'azione della luce.

Io non ho riscontrato cambiamenti di forma nei granuli interni che si presentano in genere con un aspetto tondeggiante sia nelle retine tenute all'oscuro, che in quelle le quali hanno subìto l'azione della luce; e neanche mi son potuto persuadere dell' ingrandimento degli spazi, entro i quali stanno i granuli interni e le cellule gangliari. Questi ultimi facilmente appaiono molto ingranditi per l'allontanamento delle fibre del nervo ottico dalle cellule gangliari; ma questo fatto si osserva anche nelle retine di rane tenute al buio, e dove i preparati sono ben conservati, questi spazi si vedono assai piccoli, tanto nelle retine tenuto all'oscuro, quanto in quelle, che furono esposte allo stimolo luminoso, perchè le cellule gangliari li riempiono quasi totalmente.

Le cellule gangliari hanno in genere scarsissimo il protoplasma, sicchè è molto difficile constatare se vi avvengono per azione della luce dei cambiamenti di forma, i quali in ogni modo debbono essere molto insignificanti. Nei nuclei che sono invece sempre molto grandi, non ho potuto notare alcun cambiamento.

Le fibre del nervo ottico sono così sottili che è impossibile misurarne lo spessore anche con i più forti ingrandimenti, sicchè il preteso loro ispessimento per azione della luce, che sarebbe stato osservato dal Denissenko, sfugge ad un controllo preciso, e l' impressione mia è che non esista.

Concludendo dunque questa prima parte del mio lavoro, io debbo ritenere, che le sole modificazioni morfologiche, le quali si possono osservare con sicurezza nella retina dei pesci e degli anfibi per azione della luce e dell'oscurità, si producono nell'epitelio pigmentato, nello strato dei coni e dei bastoncelli e nello strato esterno dei granuli. Queste sono:

A) Per azione della luce.

1. La migrazione del pigmento entro i prolungamenti delle cellule epiteliali, fin presso la membrana limitante esterna, la contrazione della cellula epiteliale, e la diminuzione della quantità totale del pigmento.

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2. L'accorciamento del membro interno dei coni.

3. Un assottigliamento dei membri interni dei bastoncelli della rana dovuto alla pressione degli elissoidi dei coni, che sono stati tirati vicino alla membrana limitante esterna.

4. L'allungamento dei granuli esterni della rana.

B) Per azione dell'oscurità.

1. La migrazione del pigmento entro il corpo della cellula epiteliale, l'allungamento della cellula epiteliale medesima, e l'aumento della quantita totale del pigmento.

2. L'allungamento dei membri interni dei coni.

Tutti gli altri cambiamenti di forma, che sono stati descritti, se pure esistono, debbono essere affatto insignificanti, e trovano una spiegazione più razionale, sia nelle differenze che esistono fra i medesimi elementi retinici di uno stesso occhio, ed ancor più fra quelli provenienti da occhi di due animali differenti; sia nelle alterazioni che facilmente si producono in un tessuto così delicato, per le varie manipolazioni alle quali deve essere sottoposto, e specialmente per opera dei liquidi fissatori.

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SPIEGAZIONE DELLA TAVOLA.

N. B. - Tutte le figure sono state disegnate sopra preparati fissati in liquido di Flemming e colorati con safranina e acido picrico. — (Obb. apocrom. 3 mm. Reichert, oculare 6 compens., lunghezza del tubo 160 mm.)

Fig. 1. — Da una sezione perpendicolare della metà temporale di una retina di un *leuciscus aula B.*, del peso di 15 gr., tenuto per 24 ore allo scuro. Distanza di 1 millimetro dall'ingresso del nervo ottico.

Fig. 2. — Da una sezione perpendicolare della metà temporale di una retina di un *leuciscus aula B.*, del peso di 15 gr., tenuto per 6 ore alla luce solare diretta. Distanza di 1 mm. dall'ingresso del nervo ottico.

Fig. 3. — Da una sezione perpendicolare della metà temporale della retina di un *leuciscus aula B.*, tenuto per due minuti alla luce solare diretta, dopo essere stato per 24 ore allo scuro. Distanza di 1 mm. dall'ingresso del nervo ottico.

Fig. 4. — Da una sezione perpendicolare della metà temporale della retina di un *leuciscus aula B*, tenuto per 10 minuti alla luce, dopo essere stato per 24 ore allo scuro. Distanza di 1 mm. dall'ingresso del nervo ottico.

Fig. 5. — Da una sezione perpendicolare della metà temporale della retina di un *leuciscus aula B.*, tenuto per 1 ora alla luce solare diretta, dopo essere stato per 24 ore allo scuro. Distanza di 1 mm. dall'ingresso del nervo ottico.

Fig. 6. — Da una sezione perpendicolare della metà temporale della retina di un *leuciscus aula B.*, tenuto per 5 minuti allo scuro, dopo essere stato per 5 ore alla luce solare diretta. Distanza di 1 mm. dall' ingresso del nervo ottico.

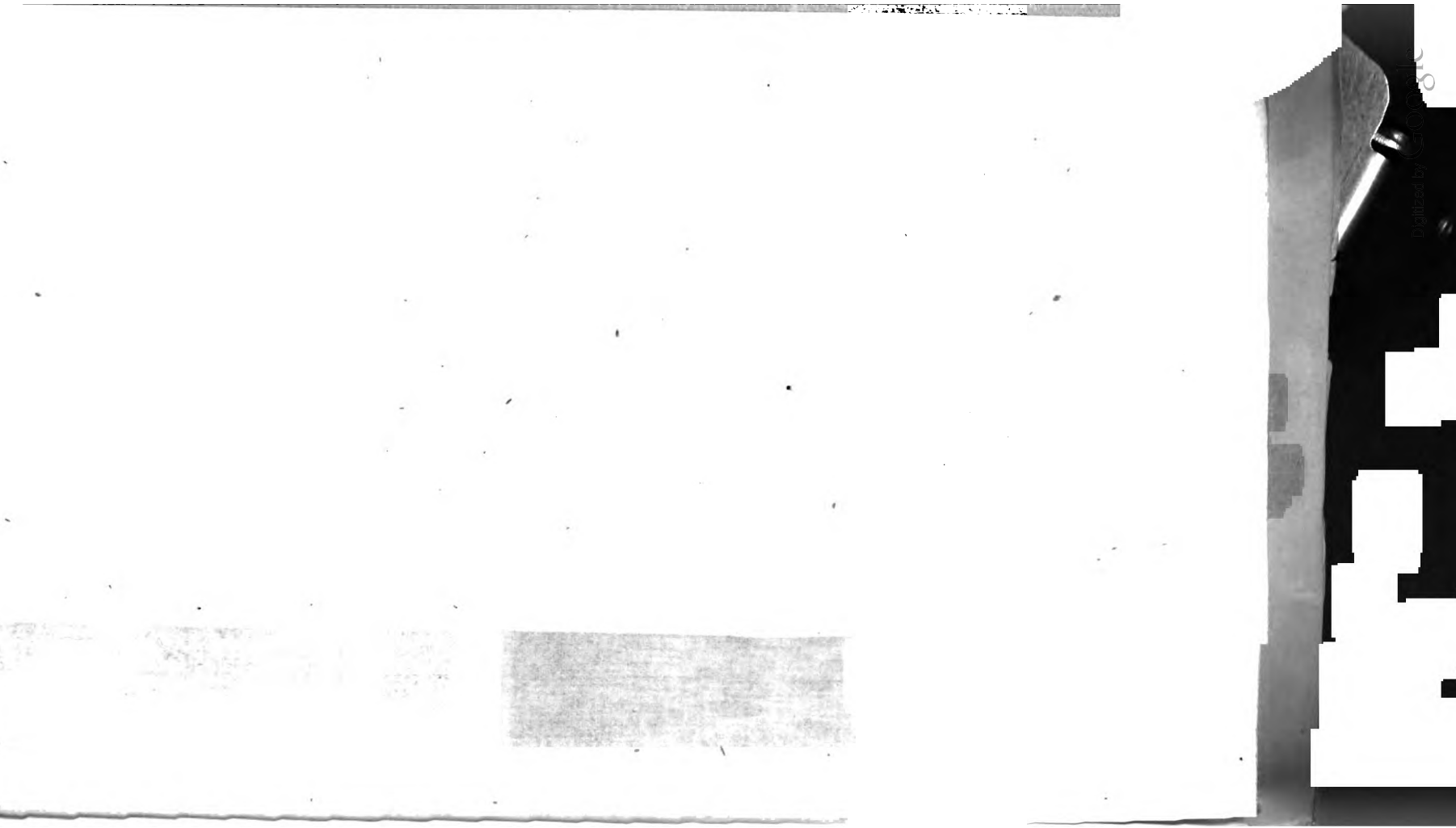
Fig. 7. — Da una sezione perpendicolare della metà temporale della retina di un *leuciscus aula B.*, tenuto per 20 minuti allo scuro, dopo essere stato per 5 ore alla luce solare diretta. Distanza di 1 mm. dall'ingresso del nervo ottico.

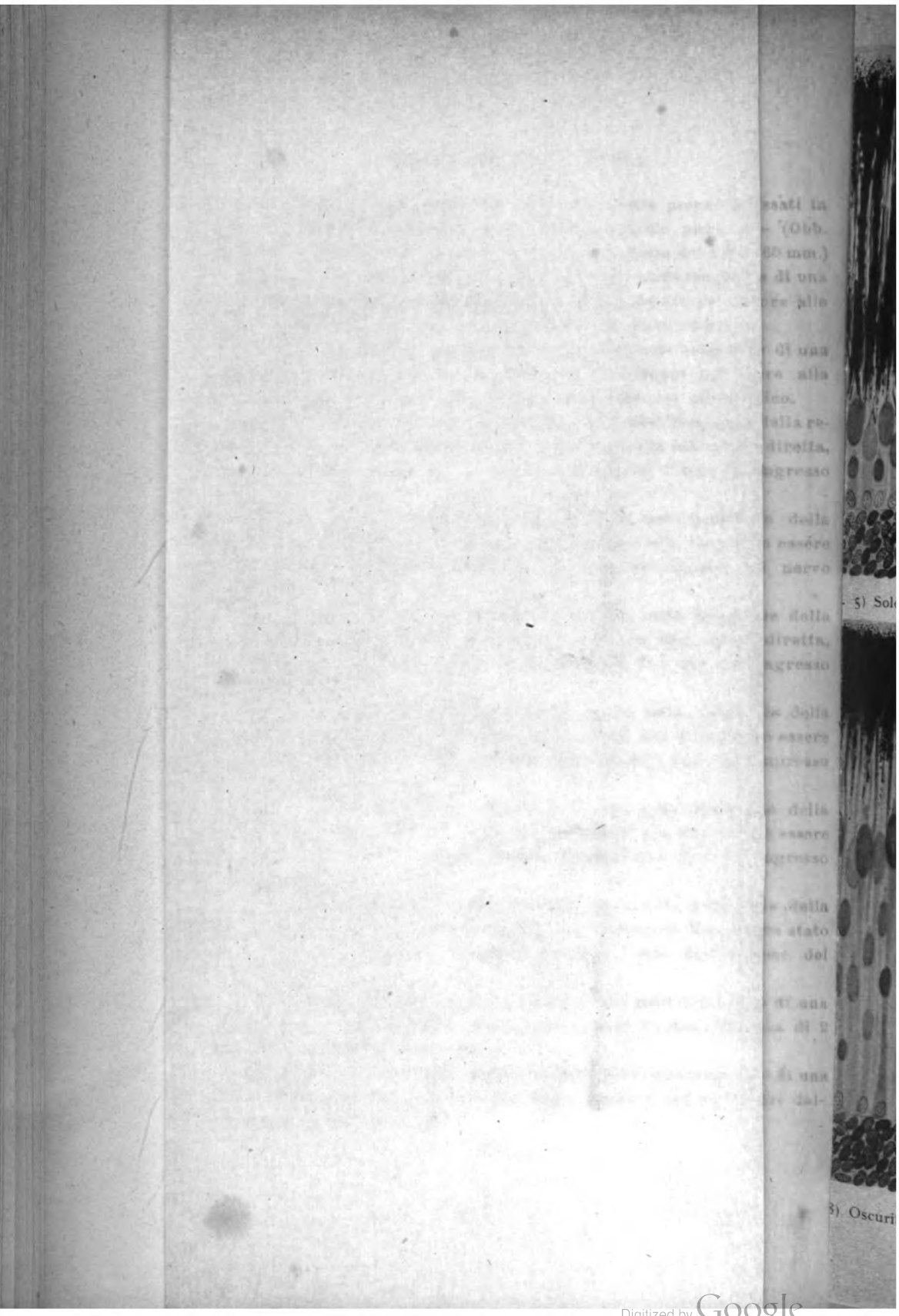
Fig. 8. — Da una sezione perpendicolare della metà temporale della retina di un *leuciscus aula B.*, tenuto per 1 ora allo scuro, dopo essere stato per 5 ore alla luce solare diretta. Distanza di 1 mm. dall'ingresso del nervo ottico.

Fig. 9. — Da una sezione perpendicolare della metà temporale di una retina di rana tenuta per 6 ore alle luce solare diretta. Distanza di 2 mm. dall'ingresso del nervo ottico.

Fig. 10. — Da una sezione perpendicolare della metà temporale di una retina di rana, tenuta per 24 ore allo scuro. Distanza di 2 millimetri dall' ingresso del nervo ottico.

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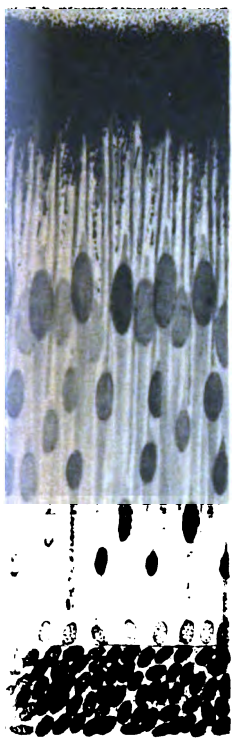


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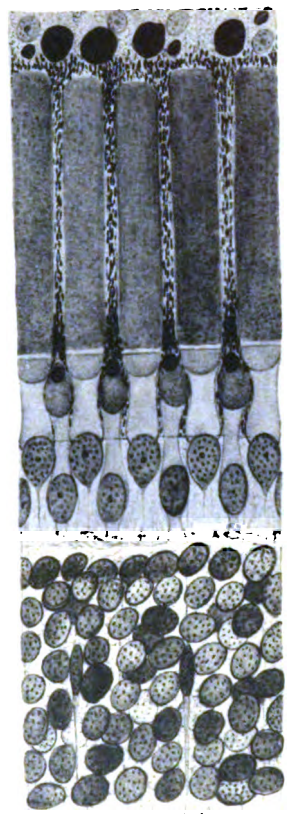
CHIARINI - Cambiamenti morfologici della retina alla luce e all'oscurità

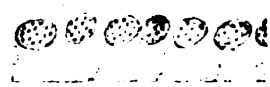


5) Sole 1 ora

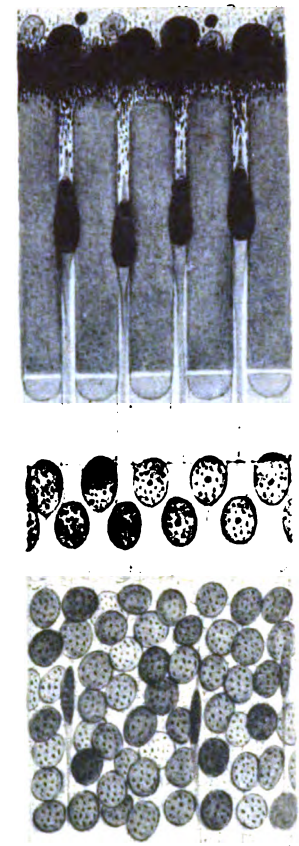


3) Oscurità 1 ora





(Fig. 9) Sole - 6 ore





(Fig. 10) Oscurità 24 ore

E. BATTISTI - ROMA

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— *Sonderabdruck.* —
Zeitschrift für wissenschaftliche Mikroskopie und für mikroskopische Technik
Band XIV, 1897, p. 175—177.

Der Rundschneidediamant, eine Vorrichtung zur Herstellung kreisrunder Glasplatten.

Von

Dr. C. J. Cori,
Privatdocent der Zoologie und vergleichenden Anatomie an der Deutschen Universität Prag.

Hierzu ein Holzschnitt.

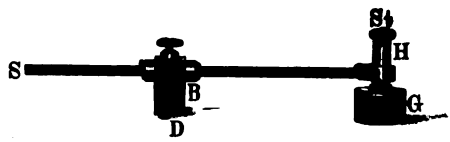
In wissenschaftlichen Laboratorien braucht man häufig runde Glasplatten zum Zudecken von Glasgefässen, welche lebende Thiere oder Präparate enthalten. Die Herstellung solcher runder Glasscheiben überlässt man gewöhnlich dem Glaser und muss dann aber nicht selten für das Schneiden einer einzigen Platte mehr bezahlen als der Glaswerth derselben beträgt. Dies ist dadurch bedingt, dass es gegenwärtig keinen einfachen handlichen und zugleich billigen Apparat für den genannten Zweck gibt. Es dürfte daher vielleicht nicht unerwünscht sein, wenn im Nachfolgenden eine solche Vorrichtung beschrieben wird, mit Hülfe deren man sich im Laboratorium aus Abfallglas um einen geringen Betrag den nöthigen Vorrath an runden Glasplatten ohne Mühe selbst herstellen kann. Ausserdem hat dieser Apparat gegen die bisher üblichen complicirten und zugleich theueren Rundschneidediamanten den Vortheil, dass er in Folge seiner Kleinheit auf Forschungsreisen bequem mitgeführt werden kann.

Der Rundschneidediamant, wie wir diese Vorrichtung nennen wollen, ist nach dem System eines Stangenzirkels gebaut. Es sind an demselben im wesentlichen folgende Theile zu unterscheiden:

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die Fixirungsvorrichtung, ferner eine prismatische Schiene, welche in einer verticalen Achse um die Fixirungsvorrichtung drehbar ist und endlich der in einem prismatischen Metallblock gefasste Diamant, der sich an der Schiene verschieben lässt.

Die Fixirungsvorrichtung besteht aus einem kleinen Holzcylinder *G* von 2 cm Durchmesser, dessen Grundfläche gerieft ist und mit Klebwachs, d. i. einem Gemisch von weissem Wachs und venetianischem Terpentin, bestrichen wird. Auf der oberen Fläche des Cylinders ist im Centrum ein verticaler Stift *St* befestigt. An letzterem sitzt eine Hülse *H*, mit der um eine horizontale Achse beweglich die genannte 25 cm lange Schiene *S* verbunden ist. Der Metallblock *B* trägt an seiner Grundfläche den Diamanten; ferner besitzt er eine Führung für die Schiene *S*, auf welcher er durch eine Schraube festgestellt werden kann.



Wenn es sich nun darum handelt, eine runde Glasplatte zu schneiden, so wird zunächst der Holzcylinder *G* mit seiner mit Klebwachs bestrichenen Haftfläche an die Glasplatte angedrückt, so dass er, wenn die Consistenz des Wachses die richtige ist, ziemlich fest auf derselben haftet. Ein Erwärmen des Wachses, von welchem nur eine geringe Menge nöthig ist, unterstützt das Anhaften wesentlich. Das Schneiden selbst führt man in der Weise aus, dass man mit dem Zeigefinger der linken Hand die Fixirungsvorrichtung an die Glasplatte fest andrückt, während die rechte Hand mit dem Diamanten einen Kreisschnitt in einem Zuge ausführt. Wenn der Diamant einen guten Schnitt gemacht hat, so springt dieser entweder selbst durch oder man braucht dann nur durch Andrücken eines zugespitzten Holzes von der Gegenseite gegen den Schnitt denselben zum Durchspringen zu bringen. Ist dies in der ganzen Peripherie geschehen, so ist es noch nothwendig, den Rand der Glastafel durch einige radiäre Schnitte zu zerschneiden, worauf ohne Mühe die runde Glasplatte herausgebrochen werden kann. Die scharfen Ränder derselben lassen sich auf einem Schleifstein oder einer Sandstein- oder Eisenplatte mit Kiessand und Wasser sehr rasch abschleifen.

Das Schneiden mit einem gewöhnlichen Diamanten ist zwar leicht auszuführen, doch muss es immerhin gelernt werden. Dies gilt in gleicher Weise von dem hier beschriebenen Rundschneidediamanten. Es ist vor allem nöthig zu beachten, dass man in einem Zuge den Dia-

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Letzteres geschieht besonders beim Schneiden ganz kleiner Glasplatten dann, wenn man die Führung des Diamanten am äusseren Ende der Schiene S vornimmt, weil man beim Aufdrücken wie mit einem Hebel die Haftplatte abreisst. Man soll also den Diamantendirect bei seiner Fassung führen. Mit Rücksicht auf diesen Umstand ist für die Anfertigung von Glasplatten mit kleinem Durchmesser eine extra kurze Schiene dem Apparate beigegeben. Den Schnitt beginnt man am besten von der Stellung aus, wie dies in der umstehenden Figur ersichtlich ist.

Mitunter ist es sehr erwünscht, in eine Glasplatte ein kreisförmiges Loch zu machen. Dies kann man mit dem beschriebenen Apparat in folgender Weise erreichen. Man schneidet zunächst den Kreis von dem gewünschten Durchmesser und sorgt dafür, dass der Schnitt in seinem ganzen Umfang durchspringt. Da sich die nun geschnittene runde Platte nicht auf einmal aus der Scheibe, in welche das Loch gemacht werden soll, herausnehmen lässt, so ist es nothwendig, die runde Platte in 4 bis 6 Sectoren zu zerschneiden. Wenn die hierzu geführten Schnitte alle durchgesprungen sind, so kann man durch leise Schläge mit einem kleinen Hammer gegen die Glasplatte, welche auf einem spitzen Metallgegenstand aufliegt, die einzelnen Sectoren zertrümmern und so entfernen. Bei einiger Uebung fällt es nicht schwer, auf die angegebene Weise beliebig grosse und runde Löcher in Glasplatten herzustellen. Solche Scheiben mit kreisrunden Löchern können statt Filtrirtassen Verwendung finden, auch eignen sie sich als Glaszellen zum Einschluss von Dauerpräparaten und dergleichen.

Den completen Rundschneidediamant liefert Herr Josef Kettner, Mechaniker an der deutschen technischen Hochschule in Prag, zum Preis von 4 fl. 50 kr. bis 5 fl.

Prag, den 16. Juni 1897.

[Eingegangen am 17. Juni 1897.]



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Band XIV, 1897, p. 178—184.

Ein horizontal fischendes Schliessnetz.

Von

Dr. C. J. Cori,

Privatdocent der Zoologie und vergleichenden Anatomie an der Deutschen Universität Prag.

Hierzu drei Holzschnitte.

Bei faunistischen und planktonischen Studien, welche ich in den Sommerferien 1894, 95 und 96 in dem nahezu 200 Meter tiefen Traunsee in Oberösterreich ausführte, erschien mir mit Rücksicht auf die Lösung gewisser Fragen, nebst der Anwendung eines einfachen Schwebenetzes, auch die eines Schliessnetzes sehr wünschenswerth zu sein, um Fänge in beliebigen und genau bestimmten Tiefen machen und so die Gesetze der Vertheilung der Lebewesen in dem genannten Seebecken feststellen zu können. Die gebräuchlichen Schliessnetze entsprachen aber aus dem Grunde nicht vollkommen den Anforderungen, weil sie entweder recht complicirt gebaut und in Folge dessen zu kostspielig sind, oder weil sie nur eine bestimmte und kurze Zeit fangend durch das Wasser bewegt werden können. Diese Umstände gaben die Veranlassung, die Construction eines Schliessnetzes,[1] das einerseits beim Fangen beliebig lange offen gehalten werden kann, anderseits um einen bescheidenen Preis herzustellen ist, selbst zu versuchen.

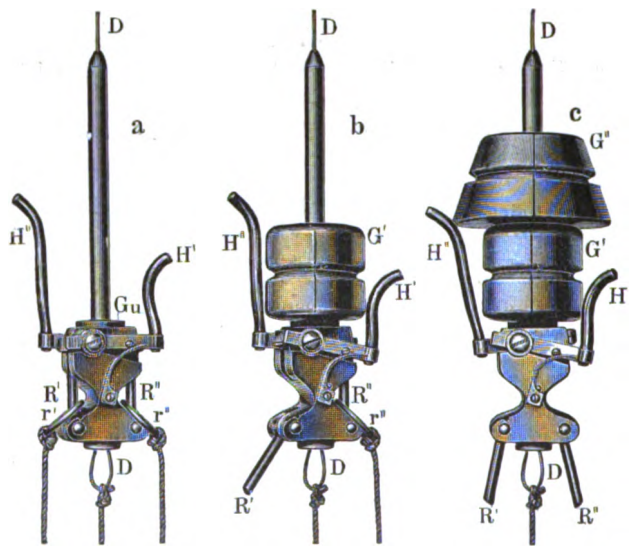
Das im Nachfolgenden beschriebene Schliessnetz besteht aus einem metallenen Netzgestell, dem Netzrahmen (Figur 2) und einer sogenannten Auslösevorrichtung (Figur 1), welche das Oeffnen und Schliessen des Netzes besorgt.

[1]) Dieses Schliessnetz war bereits auf der Ausstellung der Versammlung Deutscher Naturforscher und Aerzte, welche im September des Jahres 1894 in Wien tagte, ausgestellt und wurde auch in der Zoologischen Section dieses Congresses demonstrirt.

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Die Herstellung des Schliessnetzes wurde wesentlich dadurch erleichtert, dass bereits eine fertige Auslösevorrichtung, welche Herr Prof. Hatschek schon vor Jahren ebenfalls für ein Schliessnetz construirte, vorlag, so dass nur noch die Construction eines entsprechenden Netzrahmens erübrigte.

Die Auslösevorrichtung (Figur 1), welche zunächst beschrieben werden soll, wird in Verbindung mit dem Netzrahmen, an einem Klaviersaitendraht oder Kupferkabel befestigt, in die Tiefe versenkt (Figur 3). Sie besteht im wesentlichen aus einem prismatischen



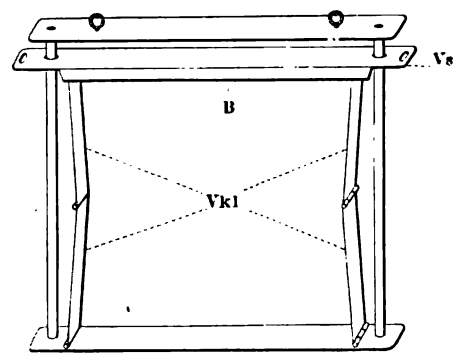
1.

Metallblock mit zwei seitlichen Auskehlungen, welche mit je einem Verschlussriegel zum Zwecke der Fixirung der Aufhängeschnüre des Netzrahmens versehen sind (Figur 1, R', R''). Die genannten Riegel sind einerseits um horizontale Achsen nach unten umklappbar, anderseits können sie durch Sperrhebel (Figur 1, H', H'') derart festgestellt werden, dass sie die Auskehlungen seitlich verschliessen. Zur Auslösung der Riegel dienen zwei Fallgewichte, welche man an dem Aufhängedraht herabgleiten lässt und die durch Aufschlagen auf die Sporen der Sperrhebel die Riegel R' und R'' frei geben (Figur 1, *b* u. *c*, G', G''). Die Fallgewichte, wie auch die Sporen der Sperrhebel sind verschieden gross und lang, so dass jedes

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Gewicht nur auf einen bestimmten Hebel wirken kann. Jedes Gewicht besteht aus zwei Hälften, um es leicht an dem Anhängedraht des Netzes anbringen oder abnehmen zu können. Durch ein Stück Draht, welches in einer Rinne um das Gewicht gelegt wird, werden die beiden Hälften fest mit einander verbunden. Damit die Fallgewichte beim Aufschlagen auf die Auslösevorrichtung und auf die Hebel eine bessere Führung erhalten, ist in dem prismatischen Metallblock eine 10 cm lange, oben conisch endigende Röhre befestigt. An der Basis der letzteren befindet sich eine Gummischeibe, welche die Aufgabe hat, die durch das Auffallen der Gewichte erzeugte Erschütterung zu dämpfen.

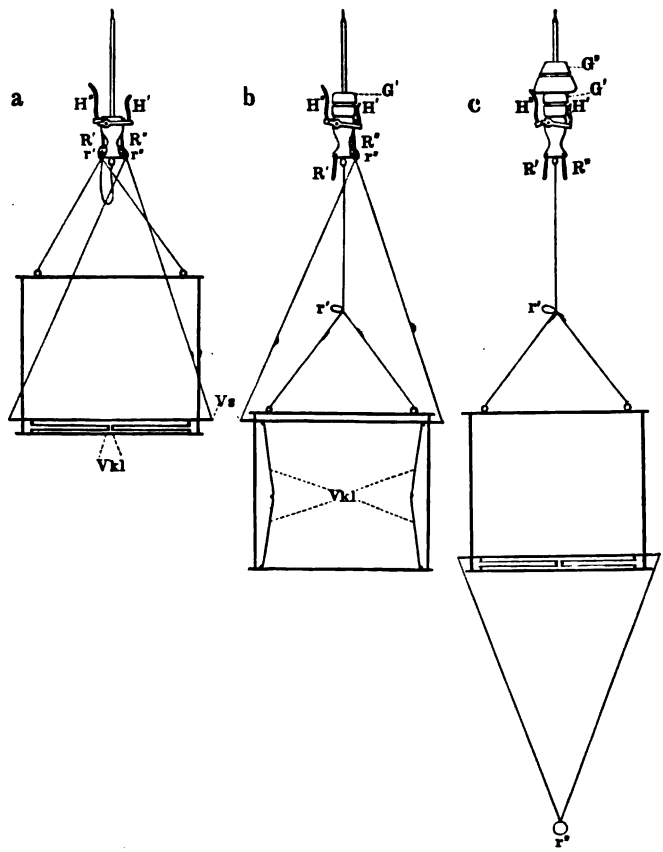
Der Netzrahmen, welcher eine quadratische Form besitzt, ist aus zwei horizontalen 30 cm langen Bandeisen und zwei verticalen Rundeisen gefertigt; letztere dienen einer ebenfalls aus Bandeisen hergestellten Schiene, Verschlussschiene genannt, zur Führung (Fig. 2, *Vs*). An dieser Verschlussschiene und an dem unteren Querstabe des Rahmens ist der Netzsack angenäht. Das Oeffnen des Netzes wird nun dadurch bewirkt, dass man die Verschlussschiene in die Höhe zieht, das Schliessen hingegen dadurch, dass man letztere fallen lässt. Damit sich die seitlichen Theile des Netzsackes beim Schliessen zur Erzielung eines prompten Verschlusses in Form von zwei Falten zwischen die Verschluss- und die untere Querschiene legen, ist noch eine an die beiden Verschlussstücke mit Charniren versehene Klappvorrichtung angebracht, an welche die seitlichen Theile des Netzsackes angenäht sind. Diese Klappvorrichtung besteht jederseits aus einem Paar von Bandeisenstücken, welche sich beim Schliessen in Form von zwei horizontal gestellten mit den Spitzen gegen einander gekehrten ≻ ≺ auf einander legen (Figur 2, *Vkl.*). Zur vollkommenen Sicherung des bereits geschlossenen Netzes ist an der Verschlussschiene ein vorspringendes, die Verschlusstheile deckendes Blech (Figur 2, *B*) befestigt.



2.

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Die Netzhose hat die bekannte Form eines ungefähr ein Meter langen Sackes und besteht in dem vorderen Drittel aus fester Leinwand, während der hintere Abschnitt aus Müllerseidenbeutelgaze No. 14 hergestellt ist. An dem Uebergang dieser beiden Stoffsorten ist ein



3.

aus spanischem Rohr hergestellter Reif eingenäht. In das offene Ende des Netzsackes kann ein weithalsiges Pulverglas von $^1/_2$ Liter Inhalt, in welchem sich die gefangenen Thiere ansammeln, eingebunden werden. Um die beistehenden Figuren nicht zu compliciren, ist die Netzhose nicht mit dargestellt worden.

Die Verbindung des eigentlichen Netzes mit dem Drahte oder

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Kabel resp. der Auslösevorrichtung wird durch Schnüre[1] aus Kuhhaaren bewerkstelligt, welche gegenüber den hanfenen Schnüren den Vortheil haben, sehr dauerhaft zu sein und im Wasser ihre Länge nicht zu verändern. Es könnten statt dieser Schnüre wohl auch Ketten in Verwendung gezogen werden. Zunächst erscheint der Netzrahmen knapp unterhalb der Auslösevorrichtung an dem Ende des Drahtes durch eine Schnur befestigt, welche gegabelt und mit den beiden Gabelenden an zwei Oesen der oberen horizontalen Querschiene des Netzrahmens angeknüpft ist (Figur 3 *c*). An der Gabelung besitzt diese Schnur einen Ring (r') der in einem so grossen Abstand von der Auslösevorrichtung an der Schnur angebracht ist, als die Höhe des Netzrahmens beträgt. An den Enden der beweglichen horizontalen Verschlussschiene ist ferner eine zweite Schnur befestigt, welche in der Mitte ihrer Länge ebenfalls einen Ring (r'') trägt.

Zum Zwecke des Fischens muss das eigentliche Netz, bevor es in die Tiefe gelassen wird, mit der Auslösevorrichtung in der Weise in Verbindung gebracht werden, wie dies in der schematischen Zeichnung Figur 3 *a*, *b*, *c* dargestellt ist.

Zunächst befindet sich das Netz in derjenigen Anordnung, wie es die Figur 3 *c* zeigt. Das Netz ist geschlossen und lediglich durch die an dem fixen Netzrahmen befestigte Schnur an dem Draht oder Kabel aufgehängt. Nun wird der Ring r'' der an der Verschlussschiene *Vs* angebrachten Schnur durch den Riegel R'' in der betreffenden Auskehlung der Auslösevorrichtung festgestellt (Figur 3 *b*); das Netz ist hierdurch geöffnet. Hierauf bringt man den Ring r' in die Auskehlung, welche durch den Ring R' geschlossen wird; der fixe Netzrahmen wird dadurch soweit gehoben, dass das Netz wieder geschlossen ist (Figur 3 *a*). In diesem Zustande erscheint das Netz zum Fange vorbereitet. Soll es nun, nachdem es so geschlossen in eine bestimmte Tiefe versenkt wurde, geöffnet werden, so lässt man zuerst das kleinere Gewicht G' an dem Draht hinabgleiten, welches durch Aufschlagen auf den Hebel H' den Ring r' freigiebt, wodurch sich das Netz öffnet (Figur 3 *b*). Es kann jetzt das Netz beliebig lange fangend durch das Wasser gezogen werden. Das Schliessen des Netzes besorgt dann das grössere Gewicht G'' durch Freimachen des Ringes r'' der Verschlussschienenschnur (Figur 3 *c*).

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[1]) Solche Kuhhaarschnüre verwenden die Fischer der Salzkammergutseen bei ihren Fischereigeräthen.

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Zum Hinablassen des Netzes wurde ein Stahldraht, ein sogenannter Klaviersaitendraht von 1·5 mm Stärke verwendet, welcher auf einer Handkurbel aufgewickelt war und der über eine einfache Zählvorrichtung lief. Da aber der Stahldraht mannigfache Nachtheile hat, wie z. B. die Neigung zum Verrosten und Schleifenbildung, ferner da er in Folge seiner starken Federung leicht aus den Rollen der Aufwinde- und Zähl-Vorrichtung herausspringt, so dürfte sich wohl für den gleichen Zweck ein dünner Kupferkabel, der geschmeidig ist und sich daher bequemer handhaben lässt, mehr empfehlen.

Mit dem im vorhergehenden beschriebenen Netz habe ich im Traunsee zahlreiche Fänge hauptsächlich während der Monate Juli, August und September, aber auch in anderen Monaten wie December, Januar, Februar, März gemacht und konnte mich hierbei stets davon überzeugen, dass es vollkommen zufriedenstellend arbeitete. Diese Planktonstudien sollen noch ihre Fortsetzung erfahren, und es mögen daher im Vorliegenden nur in aller Kürze die wichtigsten Resultate, die mit Hülfe des beschriebenen Schliessnetzes erzielt wurden und die anderen Orts noch mitgetheilt werden sollen, hervorgehoben werden:

1) Das Plankton wurde in dem genannten Seebecken nicht gleichmässig vertheilt gefunden.

2) Das Plankton zeigte eine ausgesprochene Schichtung.

3) Das Plankton wies eine Zone grösster Dichte auf.

4) In der kalten Jahreszeit liegt diese Zone tiefer, in der warmen Jahreszeit höher.

5) Die Schichtung des Plankton zeigt Tagesschwankungen.

Es mögen schliesslich noch die Kosten, um welche das beschriebene Schliessnetz hergestellt wurde, angeführt werden. Den Netzrahmen hat ein Dorfschmied um den Betrag von 2 fl., die Aufwindevorrichtung um 3 fl. in vollkommen zufriedenstellender Weise ausgeführt, die Auslösevorrichtung kostete als erstes Modell 15 fl., dürfte aber in Zukunft billiger zu beschaffen sein. Zur Herstellung der Netzhose wurden 5 fl. für den Ankauf von Seidenbeutelmüllergaze verausgabt und 250 Meter Stahldraht kosteten 5 fl. Als Zählvorrichtung zur Feststellung der Tiefe, in welcher gefischt wird, dürfte sich in bester Weise ein Tourenzähler eignen, wie solche jetzt vielfach beim Zweirad in Anwendung kommen und der für wenige Gulden zu haben ist. Somit würde man ein solches Schliessnetz für den Gebrauch in Tiefen bis zu 250 Meter um circa 30 bis 35 fl. her-

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stellen können. Für die Verwendung im Meere müsste wohl bei der Construction des Netzes Eisentheile vermieden und statt dessen vernickeltes Kupfer oder Messing benutzt werden, wodurch sich die Kosten um einen geringen Betrag erhöhen würden.

Die Lieferung des oben beschriebenen Schliessnetzes übernimmt Herr Josef Kettner, Mechaniker an der deutschen technischen Hochschule in Prag I, Husgasse.

Prag, den 12. Juni 1897.

[Eingegangen am 14. Juni 1897.]

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Band XIV, 1897, p. 184—189.

Ein Schlammsauger.

Von

Dr. C. J. Cori,

Privatdocent der Zoologie und vergleichenden Anatomie an der Deutschen Universität Prag.

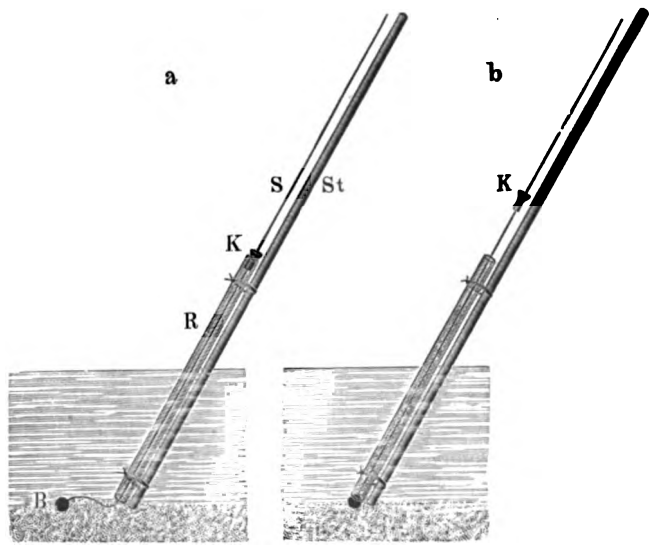
Hierzu drei Holzschnitte.

Zur Gewinnung der limnetischen Fauna für Untersuchungszwecke bedient man sich meist eines einfachen Netzes aus Müllerseidengaze, während eine andere, unter dem Namen Schlammsauger bekannte Vorrichtung nicht im allgemeinen Gebrauch ist, obzwar mit Unrecht, da gerade das letztgenannte Instrument in seiner Wirkungsweise das Netz ergänzt. Da es nun sehr wünschenswerth erschien, auch die reiche Schlamm-Fauna der Umgebung Prags dem Zoologischen Institute zu Nutze zu machen, so war hierdurch die Veranlassung zur Construction des im Nachstehenden beschriebenen Schlammsaugers gegeben.

Die ursprüngliche einfachere Zusammenstellung des Schlammsaugers, wie sie Figur 1 *a, b* darstellt, besteht aus einer 2·5 cm weiten und 50 cm langen Glasröhre *R*, welche an dem einen Ende eines entsprechend langen Bambusstabes *St* angebunden wird. Zum Verschluss dieser Röhre dient ein Kork *K* und eine Gummikugel *B*, welche beide unter einander durch eine durch das Röhrenlumen geführte 60 cm lange Schnur verbunden sind. Soll nun mit Hülfe dieses Apparates eine Schlammprobe gewonnen werden, so wird das obere Ende der Röhre mit dem gut passenden Kork *K* verschlossen, während das untere Ende zunächst offen bleibt. So adjustirt bringt man den Schlammsauger in den Sumpf (Figur 1 *a*), wobei jedoch zu beachten ist, dass der Sauger nicht in den Schlamm hineingestossen wird, sondern vielmehr nur unter einem spitzen Winkel auf

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dem Schlammgrund aufliegt. Wenn man nun mit Hülfe einer Schnur *S*, welche an dem Verschlusskork *K* befestigt ist, denselben aus der Röhre mit einem raschen Ruck herauszieht, so entweicht die Luft aus der Röhre, während das rasch einströmende Wasser den Schlamm und die denselben bewohnenden Thiere in das Rohr hineinreisst. Durch weiteres Anziehen der Schnur verschliesst man mit Hülfe des Gummiballes *B* die untere Rohrmündung, damit beim Herausziehen der Vorrichtung der Schlamm nicht wieder ausfliessen kann. Auf diese Weise erreicht man denselben Effect, wie wenn man in be-



1.

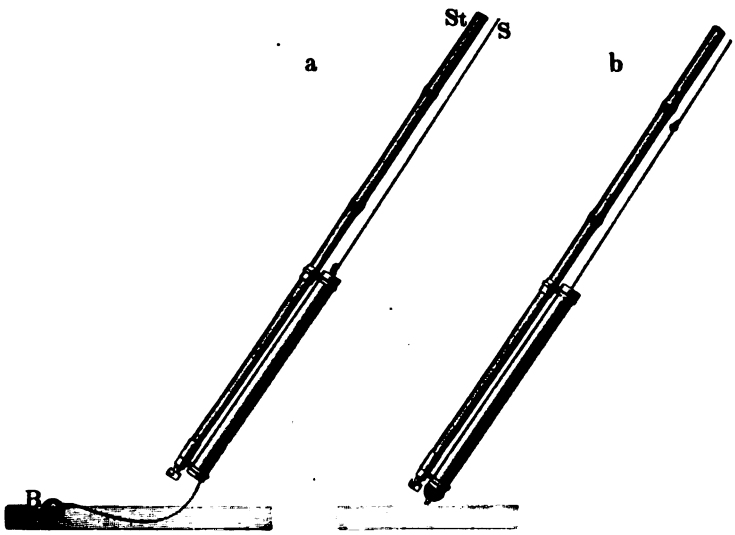
kannter Weise mit einer Glasröhre dem Aquarium eine Probe des Bodensatzes dadurch entnimmt, dass man eine am oberen Ende mit einem Finger verschlossene Röhre in das Aquariumsgefäss hält, sie hierauf schnell öffnet, um sie dann am oberen Ende wieder mit dem Finger verschlossen herauszuziehen. Durch das rasche Oeffnen wird dem Wasser ein kräftiger Auftrieb verliehen und hierbei wird gleichzeitig auch Bodensatz und die denselben bewohnenden Thiere in die Röhre hineingerissen.

Statt des leicht zerbrechlichen Glasrohres würde es sich vielleicht mehr empfehlen, für den Schlammsauger ein vom Spengler für ein billiges hergestelltes Blechrohr zu verwenden.

So einfach und leicht herstellbar diese Form eines Schlamm-

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saugers auch ist, ebenso sehr hat sie sich in der Praxis bewährt. Dies scheint hauptsächlich darauf zu beruhen, dass mit Hülfe des beschriebenen Apparates nur die oberflächlichen Schichten des Schlammes aufgesaugt werden, in welchen sich hauptsächlich Thiere aufhalten. Gleich die ersten Male wurden mit dem Schlammsauger aus Tümpeln der Umgebung Prags, welche dem Zoologischen Institute das Untersuchungsmaterial liefern, Thiere eingebracht, die bei Anwendung des Netzes nur selten zu erbeuten waren und die auch sonst als seltene Formen gelten. Unter diesen ist vor allem das grosse schöne Infusor, die Bursaria truncatella zu



2.

nennen, welche nun mit dem genannten Apparat von gewissen Oertlichkeiten der Umgebung Prags beinahe regelmässig in genügender Menge beschafft werden kann.

Auf Anregung des Herrn Prof. Hatschek wurde der im vorstehenden beschriebene Schlammsauger zu dem Zwecke, um ihn nicht bloss im seichten Wasser, sondern auch in grösseren und beliebigen Tiefen in Anwendung bringen zu können, modificirt. In dieser modificirten Form besteht der Schlammsauger[1] aus einem

[1]) Dieser Schlammsauger war bereits auf der Ausstellung der Versammlung Deutscher Naturforscher und Aerzte, welche im September des

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Messingrohr von 3·5 cm Durchmesser und 40 cm Länge, welches entweder am unteren Ende eines Netzstockes *St* durch Anschrauben befestigt wird (Figur 2), wenn es sich um seichte Gewässer handelt oder an einem Seil in eine beliebige Tiefe versenkt werden kann (Figur 3).

Das Aufsaugen des Schlammes besorgt in diesem Falle ein Kolben, welcher durch eine Schnur *S* in der Röhre *R* in die Höhe gezogen werden kann (Figur 2 *a*). Mit dem Kolben ist nun ähnlich wie im früheren Falle durch eine entsprechend lange Schnur ein Gummiball *B* in Verbindung gebracht, welcher zum Verschlusse der unteren Rohröffnung dient, sobald der Kolben bis ans obere Ende des Cylinders *R* gezogen ist und sich letzterer mit Schlamm gefüllt hat (Figur 2 *b*).

Um den Schlammsauger auch für beliebige Tiefen verwenden zu können, hat Herr Prof. Hatschek folgende Anordnung erdacht. Es wird die Schnur des Kolbens mit einem entsprechend langen Seil oder Drahtkabel, an dessen Ende ein mit einem Bleigewicht beschwerter Haken *H* befestigt ist, verbunden. In diesen Haken wird dann der Cylinder mit dem an seinem Saugende befindlichen Zapfen *Z*, der sonst zur Befestigung an dem Bambusstabe dient, aufgehängt. Ausserdem ist das obere Ende des Schlammsaugers, welches aber jetzt nach unten gekehrt ist, mit einem Gewicht *G* beweglich verbunden (Figur 3).

Wenn der so montirte Apparat, wie dies Figur 3 *a* darstellt, beim Herablassen auf den See- oder Meeresgrund mit dem Gewichte *G* aufstösst, so wird hierdurch die Röhre aus dem Haken *H* herausgehoben und fällt mit dem Saugende in den Schlamm (Figur 3 *b*). Beginnt man nachher die Vorrichtung heraufzuziehen, so wird zunächst der Kolben den Schlamm aufsaugend in dem Cylinder in die Höhe gezogen, da das Gewicht grösser ist als die Friction des Kolbens, und erst wenn sich die Röhre mit Schlamm gefüllt hat und durch den Gummiball geschlossen ist, folgt dann der ganze Apparat dem Zuge (Figur 3 *c*).

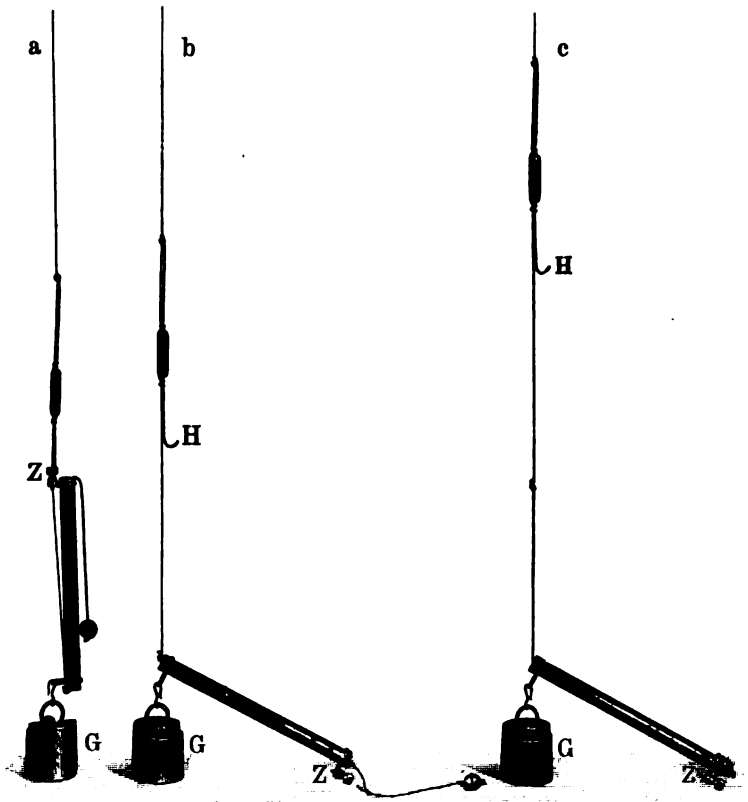
Diesen Schlammsauger in Tiefen von 100 bis 180 Meter auf seine Leistungsfähigkeit auszuprobiren, hatte ich im Traunsee in Oberösterreich Gelegenheit gehabt, und es zeigte sich hierbei, dass er stets vollkommen präcis functionirte. In den Schlammproben

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Jahres 1894 in Wien tagte, ausgestellt und wurde auch in der zoologischen Section dieses Congresses demonstrirt.

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dieses Sees, welche tieferen Stellen von 100 bis 150 Meter entnommen waren, fand sich in den Sommermonaten von Thieren nur eine kleine Kiemenschnecke, die Valvata, deren Species leider nicht festgestellt werden konnte. Letzteres Thier wurde auch in weithalsigen Pulvergläsern, welche an verschiedenen Stellen im Seegrund ausgesetzt worden waren, gefunden.



3.

Einen nicht geringen Vortheil gewährt der Schlammsauger dadurch, dass man mit demselben auch während der Winterszeit, wenn sich die Sümpfe mit einer Eisdecke bedecken, ein reiches Untersuchungsmaterial gewinnen kann. Man braucht dann nur in die Eisdecke ein Loch zu schlagen, durch welches man den Apparat auf den Grund des Sumpfes bringt.

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Der im Vorhergehenden beschriebene Schlammsauger dürfte sich wohl auch bei Tiefseeuntersuchungen verwenden lassen und scheint gegenüber der bisher geübten Methode der Gewinnung des Tiefseeschlammes mit Hülfe des Peilstockes des Tiefseelothes den Vortheil zu gewähren, dass er nur die oberflächlichen Schlammschichten, welche gerade von Thieren bewohnt werden, zu Tage fördert. Es würde sich aber für diesen Zweck empfehlen, dem Saugcylinder eine grössere Dimension zu geben, um mit jeder Lothung, die gleichzeitig hierbei vorgenommen werden könnte, eine möglichst grosse Schlammprobe zu gewinnen.

Die Lieferung des oben beschriebenen Schlammsaugers übernimmt Herr Josef Kettner, Mechaniker an der deutschen technischen Hochschule in Prag I, Husgasse.

Prag, den 15. Juni 1897.

[Eingegangen am 16. Juni 1897.]

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THE BACTERIOLOGY OF HEALTHY ORGANS.

BY

WILLIAM W. FORD, M.D., D.P.H.,

FELLOW IN PATHOLOGY, MCGILL UNIVERSITY, MONTREAL.

From the

Transactions of the Association of American Physicians

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THE BACTERIOLOGY OF HEALTHY ORGANS.

By WILLIAM W. FORD, M.D.,
FELLOW IN PATHOLOGY, MC GILL UNIVERSITY, MONTREAL.

(*From the Molson Pathological Laboratory.*)

Scientific literature is full of careful observations of those observers who have studied the various species of bacteria isolated from diseased organs and tissues in man and animals, and scattered in the literature of the past two decades are a few articles which describe the micro-organisms which have been found in the living body where no evident disease was present.

The results of these later observations have been so at variance that practically two schools of bacteriologists have developed: the one studying cultures and smears from healthy organs removed immediately after death, claiming that bacteria are normally found; and the other, using slightly different methods and finding no growth on their cultures from similar organs, attributing the successful results of its opponents to errors in technique, and coming to the conclusion that these organs are normally sterile.

Meissner,[1] early in the last decade, states that "in the living tissues of the healthy animal no bacteria capable of development are present," while Zweifel,[2] quite on the contrary, affirms that in the living tissues bacteria are always present, that, however, they are anaërobic in character, their capability of development thus depending to a larger extent upon the amount of oxygen brought to the tissues by the blood.

Hauser,[3] moreover, following the work of these two men with similar observations, found, almost without exception, that the tissues of the body were sterile, whether sections of these tissues be submitted to bacteriological examinations (Gram's method) or whether cultures be made directly from the organs on the different nutritive media.

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Welch,[4] in 1891, speaking with especial reference to the colon bacillus, states that he has isolated this organism from the internal organs of the body only where some distinct lesion of the intestinal mucosæ was present, and almost uniformly failed to find it outside the intestinal tract when no demonstrable lesion of the mucosa existed. He states, moreover, that the colon bacillus does not invade the blood and organs in the process of post-mortem decomposition.*

Among the more extended observations in this particular field of bacteriology are those of Neisser and Opitz, who have not only published the results of their own careful and painstaking researches, but have as well made accurate historical reviews of the bacteriological literature of the past thirty years, especially that portion which deals exclusively with the question under consideration.

Neisser[5] using rabbits and guinea-pigs removed the internal organs (liver, spleen and kidneys, heart, lung, mesentery) with as thorough asepsis as possible, cultivated them for *two days* on nutritive media, and finding that without exception these organs were free from bacteria at the end of this time, concluded that "under normal conditions no bacteria are present in the lymph or blood stream." After feeding different animals with cultures of various micro-organisms, however, and producing distinct lesions of the intestines, he found a large number of internal organs from which bacteria grew, either those with which the animal was treated or those that normally inhabit the intestine.

Opitz[6] removed the mesenteric glands of cattle killed at the abattoir at Breslau, carried them to the Hygienic Institute in the same city, and there, after carefully sterilizing the surface, cut out bits of these glands and cultivated them on agar and gelatin plates. Observing that in the majority of organs thus treated no bacteria grew at the end of *three days*, and that the forms isolated from the organs which decomposed were in all cases spore-bearers, especially the bacillus subtilis, the spores of which are exceedingly hard to kill, he concluded that the mesenteric glands of cattle are normally sterile, that is, that a passage of bacteria through the intestinal wall during digestion normally does not take place.

* Dr. Welch has, however, informed us—and he will, I feel assured, permit me to mention the fact—that in the course of an incompleted research in his laboratory, prior to the commencement of these investigations, it was found that the organs of healthy cats treated immediately after death by the perchloride method (to be described later) gave cultures of more than one form.



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Neither Neisser nor Opitz record any observations on organs kept longer than three days, and the latter, in the experiments described above, naturally does not eliminate the possibility of post-mortem changes, as the transportation of the organs from one institution to another, together with the means employed to sterilize the surface, made necessary by such a transportation, consume so much time that rapidly growing species of bacteria might develop, or, on the other hand, bacteria present at the time of death might be destroyed by liberated bactericidal substances; and, to say the least, Opitz is not working with tissues absolutely normal.

For now some years studies more especially on the liver, made by Professor Adami in Montreal, have revealed the presence under the microscope of minute bodies within the cells, present both in diseased states and in organs showing no special lesion, which Dr. Adami could only conclude were of bacterial origin.[7] It thus became of interest to note whether bacteria were taken up by the healthy organism, and Dr. A. G. Nicholls, Senior Demonstrator of Pathology, undertook a study of the bacteriology of the healthy rabbit. His results seemed persistently to show that there was a passage of bacteria into the normal tissues.

Lack of adequate time, however, to carry out an extended series of observations has prevented the completion of enough experiments to determine with sufficient accuracy whether this law holds true for different species of animals under different conditions, and, consequently, the work was elaborated by the Fellow in Pathology.

It is the object of the present paper to describe the experiments made, with more especial reference to the methods employed and the general results obtained than to the particular varieties of organisms isolated.

It was decided to attempt the solution of one part only of this problem, and to determine whether bacteria are normally present in the livers and kidneys of animals killed instantaneously, and not to raise the question of the path of the bacteria from their normal habitation to the organs in which they might be found, a subject which Dr. Nicholls is more especially engaged in studying at the present time.

In addition to the experiments on animals, a number of autopsy cultures were studied, and while a considerable interval of time neces-

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sarily elapsed between the moment of death and the moment of making the culture, yet the results of this set of observations seemed so much to verify the work on animals that they have been included in this paper.

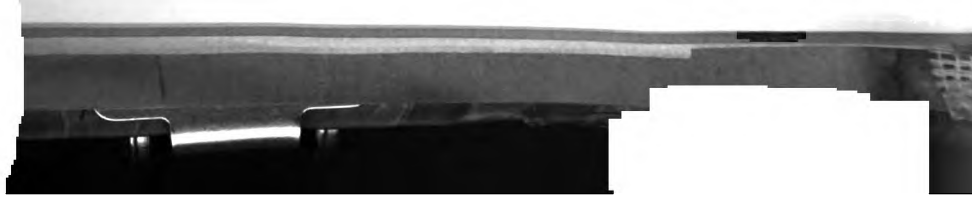
It was necessary at the outset to keep two facts especially in mind.

First, it was desirable to reproduce the conditions in which the organs normally exist, and to remove them within a time after death, so short that it could be certain that post-mortem changes were not being studied; and, secondly, cultures had to be made on a variety of media both solid and fluid, so as to avoid if possible the common experience of bacteriologists who frequently see bacteria in substances examined and yet are unable to obtain any growth from them.

In this connection may be mentioned the variance in the results of blood cultures obtained by the German and English students as compared with the French, for while the former using solid media, straight or slant, on the surface of which the blood has been smeared, have obtained in many cases no growth on their media, the latter using large quantities of fluid media, especially broth, have found bacteria in a much larger proportion of cases. It may thus be possible that the blood undiluted will exhibit a strong bactericidal power which will be lost if the blood be well diluted, and thus there is frequently a decided growth in broth, for example, as compared with a sterile culture on agar. The same law may hold true for the animal juices as well as for the blood. Therefore, animals were instantaneously killed, the organs were removed with the greatest possible care to avoid contamination; they were then preserved in gelatin, agar, or broth at the room or the body temperature, and cultures were made directly from these organs on both solid and fluid media.

It was decided, moreover, to study animals in both a condition of fasting and of full digestion to determine what changes might be caused by the state of the stomach and intestines, and bearing in mind the negative results obtained by Neisser and Opitz, it was decided to preserve the organs for such a time as to be sure that a small number of bacteria developing slowly in the animal tissues might not escape detection.

The methods employed in this work will be briefly described. The first set of animals was killed early in October of last year. Rabbits were selected, starved for twenty-four hours, killed instantaneously by a



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blow on the head, fastened to animal boards and saturated with water, to prevent the hair and fur from flying about the field of operation. The operator's hands were washed with soap and water, soaked in saturated solutions of potassium permanganate and oxalic acid, and then bichloride of mercury solution 1 : 1000 for several minutes.

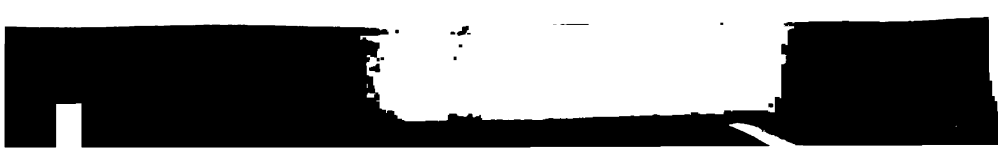
All instruments were boiled for fifteen to thirty minutes, and with separate instruments for each step of the operation, the skin was removed, a small incision in the abdominal muscles made and the kidneys and half of the liver of each animal were excised, then passed immediately into the gas jet of a Bunsen burner, where they were held in the flame with hot-flamed instruments till the outer surface was scorched or cooked to a grayish-brown. They were then transferred immediately to specially prepared gelatin bottles. These bottles were wide mouthed, furnished with ground-glass stoppers, each perforated by a small opening plugged with cotton, each bottle containing about 50 c c. of gelatin well sterilized, and the whole placed in the steam sterilizer at a temperature of 110°. At the operation the glass stoppers were removed, the smoking organ dropped directly from the flame into the melted gelatin and the glass stopper immediately replaced. The organs thus removed were preserved at the room temperature for eight days.

Cultures were made from day to day by drawing up a small bit of the organ in a sterile capillary pipette through the perforation in the glass stopper, and inoculating agar and broth-tubes. After eight days the organs were hardened in formal-Müller and sectioned and examined by the carbol-thionin and Gram's method.

From the first six organs thus prepared cultures were obtained on the fourth to the seventh day in four cases, and a similar set of organs was then removed from full-fed rabbits with the same technique and preserved in similar gelatin bottles. Positive results were obtained in four of the six organs of this series.

A third set of rabbits was killed, the organs removed (kidney and liver) with the same care, and I was particular, moreover, to flame the instruments in every case before touching the organs, and to cook the surface of the organ thoroughly in the flame. These organs were preserved in agar in the thermostat at the body temperature.

At the end of three days in this set of cultures the surface of the agar was covered with an abundant growth of bacteria, from which



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cultures were made. The organ was then removed, its surface seared by a red-hot knife, and another set of cultures taken from its interior.

The glass-stoppered bottles were now discarded in favor of small two-inch deep Petri dishes half-filled with agar, which could be managed exactly like an ordinary plate culture. Long broth-tubes of larger calibre were also employed, furnished with broken glass in the bottom, having a cruncher of glass rod passed through the cotton-plug, and containing about 100 c.c. of neutral bouillon.

Both agar and broth were sterilized in the autoclave, and at the operation they were opened for only a fraction of a second, and the hot sizzling organ passed directly into the medium.

The organ in the broth was broken up by the glass rod, which was then pulled out through the cotton-plug and the top of the tube was covered with tin-foil. Cultures could be obtained from this as from an ordinary broth-tube.

Thus, in the latter experiments, each set of animals furnished a series of cultures on broth, on the surface of the agar and from the interior of the organ.

As a later procedure the Durham modification of the Smith fermentation-tube was adopted instead of the broth-tube, and glucose broth used instead of the plain. This modification, I may say, consists in having a small test-tube inserted in a larger one, which contains sufficient broth to fill the inner tube.

With the technique and method thus described cultures have been made from the kidneys and liver of full-fed and fasting rabbits, guinea-pigs, cats and dogs, and the various bacteria isolated from them have been identified as far as possible. Especial care has always been taken to avoid contamination. For many of the experiments the instruments had been soaked for some days in a mixture of 40 per cent. formalin* and glycerin, and then boiled immediately before operation; but the surest way of securing their absolute sterility has been to heat them in the flame for several minutes before using.

In all cases the organs have been thoroughly cooked in the flame before preservation. By means of the fermentation-tube the organ preserved in broth has often been found to evolve a considerable quantity of gas, and yet the cultures from it would show only a

* Formalin is 40 per cent. solution of formaldehyde. This antiseptic was originally recommended by Dr. F. Buller.

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staphylococcus or a non-gas-producing bacillus. Smears, however, from the original tube would show various bacilli, either the colon or other forms, which were possibly overgrown by the hardier staphylococcus or some variety like mesentericus.

In many cases it has been impossible to identify the organisms isolated, so rudimentary is our knowledge of bacterial species; and while in the earlier experiments careful observations were made to determine if possible which particular variety had grown, a number of different animals being inoculated to establish this point, in the later experiments it has sufficed to describe the bacteria briefly and to separate them into rough types. I hope to describe later the character of the foregoing forms isolated in the course of these experiments.

In work of this kind the chances of contamination are many, and the greatest care must be exercised to avoid this factor. With attention to the details of asepsis, however, the organs may be removed and preserved without introducing bacteria from without into the culture media; but it is a different problem to take routine cultures from these organs from day to day over a period of several days or weeks without at times suspecting that the later growths are due to an earlier contamination. After a certain amount of experience, however, it became possible to take the cultures in the proper way, and a few organs in each series which remained sterile after being tapped for a number of times demonstrated that the bacteria which did grow in the others came from the organs themselves. To illustrate, a liver was preserved for thirty-nine days, cultures taken every third day, and yet at the expiration of this time the cultures were as absolutely free from bacteria as at first. Another organ was preserved for the same time with the same result.

The time of growth of the bacteria followed a fairly general law.

Cultures were usually obtained on the fourth, fifth, or sixth day, at which time the organ would show signs of decomposition. Rarely growths were obtained on the second and third day, but it was usually possible to foretell by the third day what particular organ would furnish a culture, both by means of the discoloration of its surface, its softness where tapped, the color it imparted to the media, and especially by gas evolved in the fermentation-tube.

As a routine measure, therefore, cultures were made every third day; the organs were preserved for fourteen days, those organs being

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declared sterile which had not decomposed by this time. Contradictions to this law were frequently noted. In a number of cases there was an abundant gas-production in the broth-tubes, and yet no growth in culture media, while in other cases the organs in agar would present every sign of decomposition, yet no bacteria could be isolated. Cultures taken immediately after removal from the body were invariably sterile, and on the first and second day usually so, though 50 c.c. flasks of bouillon, so useful in blood cultures, were employed.

The results obtained in this work have been appended to this article in tabulated form for convenience of reference, and we may now analyze the various experiments, contrasting the species of bacteria found in the different animals with the different media and under the different conditions of digestion.

The Livers and Kidneys of Rabbits.

The first twelve organs cultivated, which may be comprised in the first set, were taken from fasting and full-fed rabbits and preserved in *gelatin* at the room temperature. From eight of these cultures were obtained, including staphylococci, spore-bearing pathogenic bacilli, varieties of proteus, and a paracolon. The time of growth was often late, sixteenth to seventeenth day, and many of the bacilli were spore-bearers.

Both the late period of development and the character of the bacilli grown might be expected in gelatin cultures at the room temperature. From the four organs remaining sterile, cultures were made at intervals for many days, and yet no growth resulted.

Out of a total of twelve organs eight contained bacteria, a percentage of 66⅔. The same percentage is true of the cultures made.

In the second set, kidneys and livers of rabbits preserved in *agar*, there were fifteen organs cultivated with eighteen cultures. Bacteria were obtained in fifteen of the eighteen cultures—83.3 per cent., while thirteen of the fifteen organs grew—86.6 per cent.

This difference in percentage develops from the fact that two portions of the same liver may be cultivated on different media—for example, in broth and agar, while the kidneys in every case were saved entire. In each series the different parts of the liver have been considered as one organ, while the different kidneys from the same animal necessarily are separate organs, for while the general

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conditions governing the lymph and vascular supplies of the two kidneys are identical, yet the bacteriology of the two kidneys of the same animal may be quite different, as has been seen in many cases.

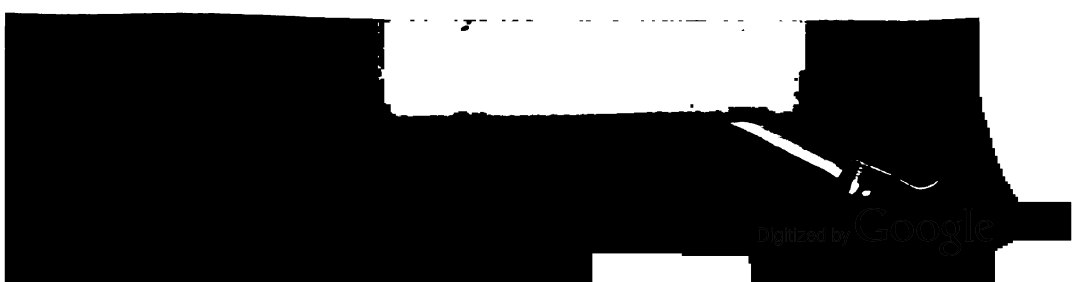
Cultures were obtained from the fasting animals in every case, the growth on the surface of the agar corresponding to that from the organ itself, and, as a rule, with that in the broth-tube. The predominant types are staphylococcus, mesentericus, colon, and paracolon in the fasting animals, while the mesentericus, staphylococcus, subtilis, and proteus grew from the full-fed animals. No culture of colon appeared, however, in the full-fed condition. The percentage of growth in this series is much higher that in any other set of animals, and illustrates possibly a condition of lowered resistance on the part of the rabbits used. They were taken from the laboratory supply which had been kept in confinement in unhygienic conditions for many weeks, and can hardly be said to represent the normal conditions of animals at liberty.

In these cultures the form appearing at the surface is often only one of the forms grown from the organ, as in Series III., Liver II., where a staphylococcus alone appeared on the surface of the agar, and in the organ there grew staphylococcus, mesentericus, and two varieties of paracolon. In Series IV. it may be noted that from Liver I. in both agar and broth the cultures were identical, while the third liver and kidneys were sterile in agar and grew staphylococcus in broth.

A number of cultures were taken immediately in 50 c.c. flasks of bouillon, all of which were sterile except the third kidney, from which grew a mould.

By accident one portion of one liver was preserved without singeing. Its duplicate was preserved in the regular manner; but from both of these organs, and from a part of the organ in broth, the same variety of mesentericus developed.

In general it may be seen that when an organ covered by agar decomposes, only one or two of the forms of bacilli present in the organ grow through the agar to the surface, and that while an organ in agar may not furnish a culture, yet the broth emulsion of another part of the same organ will. Either the breaking up of the organ liberates the bacteria more easily or the broth itself is a better medium for developing an organism. It is possible that the liberation and


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neutralization of the bactericidal substances in the juices of the organ may be especially facilitated by a fluid medium.

Livers and Kidneys of Guinea-pigs.

The third set of animals (guinea-pigs), prepared in the usual way, grew practically the same variety of micro-organisms as did the rabbits, except that here the bacillus subtilis appeared in several of the cultures.

Its presence raised immediately the question of the proper preparation of the culture media, but its consistent appearance in cultures on both agar and broth, as in Kidney III., Series V., and its isolation from both the surface of the agar and the interior of the organ, as in Kidney II., Series VI., contraindicated the suspicion that it was an interloper.

A number of sterile organs in this series also pointed to the purity of the culture medium. In the subsequent experiments, however, the Petri dishes of agar were not only sterilized in the autoclave, but tested for a week or more in the thermostat on the suspicion that the spores of the subtilis might have been present in the agar stock.

From twenty-four cultures made here definite results were obtained in eighteen cases—a percentage of 75, while from the actual number of organs cultivated (eighteen) fourteen grew (77.7 per cent.).

Livers and Kidneys of Cats.

The cats next used were more suitable than the rabbits, as they were brought from the country, 150 miles away, kept in confinement for a few days only before operation, and thus represent a thoroughly wild condition. A different variety of organisms resulted from the cultures of this set. Staphylococcus and colon persist, subtilis and mesentericus drop out, and a number of forms like proteus, megatherium, and mycoides appear, as well as a staphylococcus and two varieties of long, narrow, spore-bearing bacilli, whose growth was so slow and weak that a careful study of their characters was impossible. Anaërobic cultures in Buchner jars likewise failed to reveal their identity.

From the twenty-four cultures here made growths were obtained or definite bacteria noted in twenty cases, a percentage of 83.3; fourteen of the eighteen organs contained bacteria (77.7 per cent.).


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Livers and Kidneys of Dogs.

In the fifth set of animals the proper conditions were more nearly fulfilled than in any other experiment. The dogs were wild, had been confined only a few days; the technique of operation and the method of making cultures was better understood and handled; there was no doubt of the proper sterilization of the culture media and the Durham-Smith fermentation-tube furnished an accurate means of collecting any gas produced in a decomposing organ, thus indicating the presence of bacteria. The thorough surface cooking which the larger size of the organs permitted, and to which each organ was subjected, left no doubt of the absolute sterility of the surface.

The same flora was isolated as from the cats, especially forms like mycoides, megatherium, B. Zopfii, and many long, spore-bearing bacilli, which could be grouped together like those of the preceding set. The growth of the latter was very scanty, but the microscopical characters of the bacilli were unmistakable. Out of the twenty-four cultures here twenty-one grew (87.5 per cent.), while sixteen of the eighteen organs contained bacteria (88.8 per cent.).

Fœtal Organs.

Four organs at this time were removed from fœtal kittens and cultivated in the same way. Three remained sterile, from one grew a staphylococcus and megatherium, an apparently inexplicable result, yet the four organs were all subjected to the same procedure, especially in the sterilization of the surface.

Sterilization by Perchloride of Mercury.

At the suggestion of Dr. Welch a final set of organs was prepared with a somewhat different method, to see if possible whether any change in the procedure adopted could make any change in the actual results obtained.

Rabbits were killed, the organs were removed carefully, and then placed in dishes of bichloride of mercury 1 : 1000, and left for one hour. The dishes had previously been soaked for from two to eighteen hours in this solution. The organs were left in bichloride an hour, transferred to sterile water, and there left another hour, rinsed off with


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boiling hot sterilized water, and placed in fermentation-tubes and sterile Petri dishes, into some of which agar was subsequently poured.

By this method the bichloride of mercury forms an insoluble precipitate of albuminate of mercury on the surface of the organ, which allows complete sterilization of the surface without destruction of the micro-organisms in the interior.

Twenty cultures prepared in this way grew in thirteen cases (65 per cent.), the forms being identical with those from the rabbits originally used, mesentericus, staphylococcus, and colon being more especially present. Of the actual number of organs employed (twelve) nine showed bacteria (75 per cent.).

Examination of Sections of the Organs.

Bacteriological examination of the hardened organs was made in all cases, and the results were somewhat difficult to interpret. In many organs from which bacteria grew the forms isolated could be easily demonstrated in the sections, especially mesentericus and staphylococcus. The bloodvessels, as a rule, were filled by the bacteria. In other cases, however, the only evidence of bacteria was the appearance, under the microscope, of many granules, both extracellular and intracellular, especially in the forms of cocci and diplococci, the numbers of these bodies being relatively great in the organs from which cultures were obtained as compared with a small number of granules seen in sterile organs, and practically none in the fœtal.

The appearance of these bodies was not determined by the variety of bacteria isolated, as they were common to all the organs, whether a staphylococcus, mesentericus, subtilis, or colon was the result of cultivation, and the entire phenomenon suggested the possibility that these granules were broken up, destroyed bacteria.

Results from Autopsies (Man).

The autopsy cultures were made by means of a simple device like a potato-cutter or cork-borer, provided with a piston, which, after sterilization by heat, was pushed through the sterilized surface of the organ, and, when withdrawn, carried with it a portion from the interior of this organ, which was placed in the fermentation-tubes. These cultivations were made some hours after death—four to six hours in the earliest case—and, of course, this element of time de-

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tracts somewhat from the accuracy of the results. Twenty-six cultures were made from nine different autopsies, and in every case bacteria were grown from these cultures.

The colon, staphylococcus, and mesentericus were the common forms, together with a number of long, narrow bacilli like those seen in gangrene. No putrefactive organisms were isolated, and from the similar results of early and late cultures one is led to believe that at the time of death a few bacteria were scattered in the organs, which are capable of development and transplantation, rather than that there was a post-mortem passage of bacteria from the intestine, for example, into the organs. In several cases a pure staphylococcus was grown, yet a large quantity of gas was evolved from the original culture, smears made directly, however, showing many bacilli, which might either be the gas-producing colon or some pure anaërobe overgrown by the hardier staphylococcus. Anaërobic cultures in Buchner's jars revealed nothing further to solve this problem.

Conclusions.

There are a number of generalizations which can be made from the scrutiny of the various experiments described above, which both testify to the accuracy of these observations and explain in great part their contradiction of the conclusions of earlier investigators.

A. *Each species of animal showed its own peculiar bacteriology* quite distinct from that of other species, regardless of the conditions in which the animals were killed or in which the cultures were made.

Rabbits. From the kidneys and livers of rabbits there grew mesentericus, staphylococcus, colon, proteus, and a variety of spore-bearing pathogenic bacilli which was not isolated from any other animal. These bacteria were obtained in agar, in gelatin, and in broth, from either full-fed or fasting animals, an interval of several weeks elapsing between the cultivation of each series. The last rabbits were killed over six months after the first, the organs were preserved in a different manner, and yet the cultures were practically identical with those originally seen.

Guinea-pig. The flora from the guinea-pig was similar to that of the rabbits, and yet not identical.

There is not the same profusion of mesentericus as in the other animals, and the bacillus subtilis becomes a quite common form. A

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greater number of organs remained sterile. Staphylococci and colon appeared in about the same proportion of the cultures. Both the rabbits and guinea-pigs are herbivora, and their organs show species of bacteria fairly consistent with the food on which such animals live.

Cat. In the organs of the cats there appeared quite different forms, megatherium, mycoides, Zopfii, with an occasional subtilis and mesentericus.

Staphylococci and colon were common, but the prevalent forms were of unknown species grouped about two common types, A and B—long, straight bacilli—spore-bearers, growing with great difficulty on the ordinary media, producing gas in the culture tubes, and isolated from the majority of the organs of this series of animals.

Dog. The forms seen in dogs were similar to those in the cats. There were very few staphylococci, no colon or paracolon, and the spore-bearers of the other carnivora were here replaced by long, narrow bacilli without spores, and by broad, straight bacilli, both gas producers and difficult to cultivate. Mycoides, megatherium, and B. Zopfii continue to grow in many of the cultures.

As a rule, the carnivora, dogs and cats, showed species of bacteria quite similar to each other, yet absolutely different from the cultures obtained from the rabbits and guinea-pigs—herbivora, and these results are thus quite consistent with the difference in the food used by the animals, which would determine to a large extent the intestinal flora.

B. *Each animal used, regardless of its species, showed its distinct bacteriology.* Among the rabbits the second animal of the third series, killed fasting, showed in its organs staphylococcus, mesentericus, paracolon, and proteus, while the third animal showed staphylococcus alone in all its organs.

The first animal of the fourth series showed mesentericus and staphylococcus, while from the third animal nothing grew on agar, and only staphylococcus in broth.

Among the guinea-pigs the second animal of Series VI. showed staphylococcus and colon, while the organs of the third animal were sterile throughout.

There is a corresponding similarity in the cultures obtained in the separate dogs and cats, but possibly the last set of rabbits shows examples of this law more convincing than are seen in any other series.

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From the first animal there grew colon or paracolon in every organ, either alone or in combination with other bacilli. The cultures of all of the organs of the second animals were sterile; from the third animal there were cultures from the kidney only; while from the last there was a mixture of colon and staphylococcus.

From two animals, at least, there were no cultures. From five or six there was only staphylococcus in the broth, and from a similar number a single species, like mesentericus, grew from all the organs.

C. *The different organs of the various animals displayed the same bacteria on the different culture media.* Liver I., Series IV., grew mesentericus in both agar and broth, as well as the unsinged liver in agar; Liver I., Series VI., grew paracolon throughout, while Kidney I., Series VII., grew bacillus subtilis on all media.

D. *The different culture media furnished a variation in the species of micro-organisms quite consistent with the growths obtained from the organ itself.* The *surface* of the agar showed frequently one form only, which was isolated later from the organ in combination with other forms, while the broth developed bacteria from organs which were sterile in agar. For example, the surface of the agar in Liver II., Series III., grew staphylococcus alone, but in the organ there appeared paracolons, mesentericus, and staphylococcus. The broth cultures of Liver III. and Kidney III., Series IV., grew a staphylococcus, while the agar cultures were sterile.

It is thus evident that when an organ decomposes, covered by agar, some bacteria develop rapidly and appear on the surface either alone or before the others present in the organs can reach the same position, and it is a significant fact that in practically every organ cultivated in this series of experiments that particular species of bacterium developing on the surface of the agar was obtained later from the singed organ.

The only possible exception to this law is in the case of Kidney I., Series III., where on the surface of the agar a mesentericus appeared, with a staphylococcus, while from the organ a few days later the staphylococcus only grew.

E. *The condition of digestion exerts a decided but not a universal influence* on the species of bacteria isolated from the kidneys and livers cultivated. In many cases the colon group appears in the cultures

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from the fasting animals, but fails to do so in the full-fed condition. This is especially well illustrated in Series III. and IV., where the fasting rabbits furnished colon in three out of six organs, while the full-fed rabbits furnished none. The prevalence of the colon group was not marked sufficiently, however, to prove this law in other species of animals, while in the carnivora it was impossible to work out a corresponding law for the unknown bacteria there isolated. The actual number of sterile organs seems to be the same, whether we are dealing with the full fed or fasting.

F. As has already been pointed out, *there was in a number of cases ample microscopic evidence to prove that bacteria had developed in an organ, and yet no cultures could be grown.*

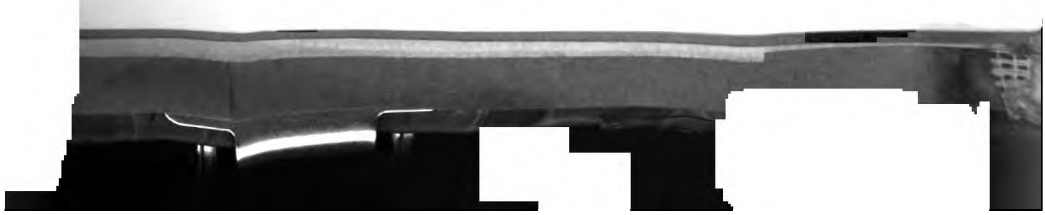
There was a production of gas in several of the Durham-Smith tubes of Series XI., yet no growth on culture media, while the portions of liver of the third animal were discolored, soft and juicy, like those which had furnished a growth of bacteria, and yet the cultures were invariably sterile.

The microscopic examination of these hardened organs showed no bacteria, but the same granular appearance as was noted in the decomposed organs.

The entire number of animals killed was 34, furnishing 93 organs and 122 cultures. Of the 93 organs, 75 furnished definite growths on the various media, a percentage of 80.6. Of the actual number of cultures made, 122, 95 grew, a percentage of 77.8.

This apparent discrepancy is explained when we remember that one portion of an organ may grow on one medium, and a corresponding portion on another medium be sterile, the broth in all cases developing more bacteria than the agar. Each set of animals as well, when analyzed separately, showed over 65 per cent. if we consider the different cultures, and over 66 per cent. if we consider the organs. While the highest percentage of organs containing bacteria was 88.6, that of cultures growing was 87.5.

In view of what has already been said, there is some evidence that the total number of organs furnishing cultures is smaller than the number of organs which might actually contain bacteria, as several decomposed organs are marked sterile because no bacteria were grown from them.



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Contamination of the cultures may be eliminated for two reasons: The utmost care was taken to remove the organs aseptically and to preserve them in sterile media in sterile dishes. The instruments were thoroughly boiled and flamed, the media were sterilized in the autoclave and then tested, the operator's hands were surgically clean, and in the actual manœuvres care was taken to prevent any possible introduction of bacteria from without. Each organ was either thoroughly singed or soaked in bichloride of mercury to sterilize the surface, and then passed immediately into the culture media prepared for it. The routine cultures were made in the most careful manner possible, and the actual preservation of several organs in the various media, gelatin, agar and broth, for from two to six weeks, and their absolute sterility at the end of this time proves that the laws of bacteriology were carefully adhered to.

Again, *the isolation of different bacteria from different species of animals, from different animals and from different organs, shows that the results of cultivation of these organs depend on actual presence of bacteria in the organs themselves, and not on the accidental introduction of germs from either the air, instruments, the hands of the operator, or the media and dishes used.*

The removal of the organ from the animal the moment after death and its transfer to the culture medium consumed less than five minutes of time, and it is thus certain that these observations represent actual living conditions.

In conclusion, why do these results contradict the results of Neisser and Opitz? The answer to this question is simple. Neisser and Opitz cultivated their organs for two and three days only, and at the end of this time declared their organs sterile. *In my cultivations at the end of three days an identical condition was determined. The cultures were sterile.* There is, then, no doubt thrown upon the facts adduced by these observers. If, however, the organs be left for several days, a week, or two weeks, bacteria will appear on the culture media with such persistence and in such profusion and specific variety as to show that they come from the actual organs and are not introduced from without.

There are two explanations for this phenomenon. On the one hand, the bacteria present in the organs may be so few in number that immediately after death they will not grow on culture media, for

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it is now being admitted that to obtain a culture of bacteria in many cases it is necessary to have them present in some slight number. As Haffkine has pointed out, the transfer of bacteria from one medium to another leads to the destruction of a certain number from alteration of environment. Thus where few are present they may all succumb.

On the other hand, the bactericidal substances in the animal juices may be so powerful in the living condition that if they be transferred with bacteria to culture media they will there exert an inhibitory action on these bacteria and render a culture sterile (especially in solid media), whereas, the same juices may lose their bactericidal power if separated from the living animal, as in the case of blood serum, thus allowing the growth and multiplication of any bacteria present in the living organ. This curiously slow development of bacteria within healthy organs removed from the body affords a most interesting problem for further elucidation.

But whatever be the explanation of the facts as a result of the experiments described in this article, it must be concluded that at least 80 per cent. (80.6 per cent.) of the livers and kidneys of healthy normal animals contain bacteria which are capable of development, provided the proper culture media be adopted, and provided that these organs be cultivated for a sufficiently long time after their removal from the animals used.

My thanks are due to Dr. Adami for his great kindness and his unfailing help and advice in this series of experiments.

SET I.

SERIES I.—*Rabbits Fasting. Organs in Gelatin Bottles.*

Rabbit A 1:			
Kidney	I.	Staphylococcus.	8th day.
Liver	I.	Pathogenic spore-bearing bacillus, No. I.	4th day.
Rabbit A 2:			
Kidney	II.	I. Pathogenic spore-bearing bacillus, No. II. II. Paracolon. III. Pathogenic spore-bearing bacillus, No. III.	7th day.
Liver	II.	0	
Rabbit A 3:			
Kidney	III.	0	
Liver	III.	Staphylococcus aureus.	7th day.



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SERIES II.—*Rabbits Full Fed. Organs in Gelatin Bottles.*

Rabbit B1:			
Kidney	I.	Pathogenic spore-bearing bacillus, No. IV.	12th day.
Liver	I.	0	Sterile after 39 days.
Rabbit B2:			
Kidney	II.	Staphylococcus.	17th day.
Liver	II.	0	Sterile after 39 days.
Rabbit B3:			
Kidney	III.	Staphylococcus.	4th day.
Liver	III.	Proteus.	
		Paracolon.	16th day.

SET II.

SERIES III.—*Rabbits Killed Fasting. Organs in Agar Bottles in Thermostat.*

		Surface.	Organ.
Rabbit C1:			
Kidney	I.	Staphylococcus.	Staphylococcus.
		Mesentericus vulgatus.	
Liver	I.	Long spore-bearing bacillus.	Staphylococcus.
		Paracolon A.	Paracolon A.
		Paracolon B.	
Rabbit C2:			
Kidney	II.	Paracolon C.	Proteus vulgaris.
		Mesentericus vulgatus.	Mesentericus vulgatus.
			Paracolon B.
Liver	II.	Staphylococcus.	Paracolon.
			Paracolon B.
			Mesentericus fuscus.
			Staphylococcus.
Rabbit C3:			
Kidney	III.	Staphylococcus.	Staphylococcus.
Liver	III.	Staphylococcus.	Staphylococcus.

Growths obtained on sixth day, previous to which the bottles were unopened.

SERIES IV.—*Rabbits Killed Full Fed. Organs in Agar Plates.*

Rabbit D1:	Surface.	Organ.	Broth tubes.
Kidney I.	Mesentericus ruber.	Mesentericus vulgatus.	Staphylococcus.
Liver I.	Mesentericus vulgatus.	Mesentericus vulgatus.	Mesentericus vulgatus.
			Mesentericus fuscus.
Rabbit D2:			
Kidney II.	0	0	Mesentericus fuscus.
Liver II.	Mesentericus vulgatus.	Mesentericus vulgatus.	Mesentericus vulgatus.
	Proteus mirabilis.	Proteus mirabilis.	Bacillus subtilis.
Rabbit D3:			
Kidney III.	0	0	Staphylococcus.
Liver III.	0	0	Staphylococcus.
Liver I.	Not singed.		
	Mesentericus fuscus.	Mesentericus vulgatus.	
	Mesentericus vulgatus.		

All cultures taken immediately in 50 c.c. bouillon sterile except kidney III., from which grew a mould. Growths obtained fourth to sixth day.

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SET III.

SERIES V.—*Guinea-pigs Killed Full Fed. Organs in Agar Plates.*

		Surface.	Organs.
Guinea-pig A 1:			
Kidney	I.	Staphylococcus. Paracolon.	Staphylococcus. Paracolon.
Liver	I.	Mesentericus vulgatus. Bacillus subtilis.	Proteus vulgaris. Mesentericus fuscus.
Guinea-pig A 2:			
Kidney	II.	0	0
Liver	II.	Staphylococcus.	Staphylococcus.
Guinea-pig A 3:			
Kidney	III.	Mesentericus ruber. Bacillus subtilis.	Mesentericus vulgatus. Mesentericus fuscus.
Liver	III.	Bacillus subtilis.	Bacillus subtilis.

Growths obtained at end of seventh day.

SERIES V.—*Guinea-pigs Killed Full Fed. Organs Preserved in Broth-tubes.*

Guinea-pig A 1:			
Kidney	I.	Mould on surface.	
Liver	I.	Not singed.	Staphylococcus.
Liver	I.	Singed.	Staphylococcus.
Guinea-pig A 2:			
Kidney	II.	Mesentericus fuscus.	
Liver	II.	Bacillus subtilis and staphylococcus.	
Guinea-pig A 3:			
Kidney	III.	Bacillus subtilis.	
Liver	III.	0	

Growths obtained at end of seven days.

SERIES VI.—*Guinea-pigs Killed Fasting. Organs in Agar Plates.*

		Surface.	Organ.	Organs in broth-tubes.
Guinea-pig B 1:				
Kidney	I.	0	0	0
Liver	I.	Paracolon.	Paracolon.	Paracolon.
Guinea-pig B 2:				
Kidney	II.	Bacillus subtilis.	Bacillus subtilis.	Staphylococcus.
Liver	II.	0	Staphylococcus.	Staphylococcus.
Guinea-pig B 3:				
Kidney	III.	0	0	Mould.
Liver	III.	0	0	Mould.

Growths obtained at end of seventh day.

SET IV.

SERIES VII.—*Cats Killed Fasting. Organs in Agar Plate.*

		Surface.	Organ.	Broth-tubes.
Cat A 1:				
Kidney	I.	0	Bacillus subtilis.	Bacillus subtilis.
Liver	I.	0	Few spores; no bacilli.	Long bacilli and spores. Variety A.

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Cat A 2:		Surface.	Organ.	Broth-tubes.
Kidney	II.	Proteus. Megatherium.	Proteus. Megatherium.	0
Liver	II.	0	Long bacilli and spores. Variety A.	Few long bacilli; no spore Variety A.
Cat A 3:				
Kidney I	I.	0	0	0
Liver	III.	0	Mesentericus. Mycoides. Megatherium.	Long, broad, thick bacil and spores. Variety B.

SERIES VIII.—*Cats Full Fed. Organs Preserved in Agar Plate.*

Cat B 1:		Surface.	Organ.	Broth-tubes.
Kidney	I.	Mould (mucor) Colon bacilli (?)	Mould. Colon bacillus.	Few bacilli and spores. Variety B.
Liver	I.	0	Staphylococcus. Colon.	Few long narrow and fe broad bacilli. Variety A
Cat B 2:				
Kidney	II.	0	Few long bacilli. Staphylococcus.	0
Liver	II.	0	Few bacilli. Spores. Staphylococcus.	Streptococcus.
Cat B 3:				
Kidney	III.	0	Fine bacilli on smear; no growth.	Few long fine bacilli. Variety A.
Liver	III.	0	Few bacilli; no growth.	Long straight bacilli; no spores. Variety B.

Gas produced in majority of broth-tubes, which were furnished with inner tub to collect it. Both Series VII. and Series VIII. anaërobic cultures negative. Growths obtained at end of seven days.

SET V.

SERIES IX.—*Dogs Full Fed. Organs in Agar.*

Dog A 1:		Surface.	Organ.	Broth.
Kidney	I.	0	0	Long straight bacilli, lik Zopfii, short bacilli an large coccoid bodie like staphylococci.
Liver	I.	0	Few long straight bacilli.	Few short straight b cilli.
Dog A 2:				
Kidney	II.	Mycoides. Megatherium.	Megatherium.	Long bacilli with spor like Zopfii.
Dog A 3:				
Kidney	III.	0	Long straight bacilli; no spores.	Few long straight bacill
Liver	III.	0	Few long straight bacilli.	Long straight bacilli lik mycoides, others lik megatherium.

Gas produced in all broth-tubes of Series IX.



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SERIES X.—*Fasting. Organs in Agar.*

		Surface.	Organ.	Broth.
Dog B 1:				
Kidney	I.	0	Staphylococcus. Mycoides.	Long straight bacilli like mycoides.
Liver	I.	0	Sporelike bodies and long straight bacilli.	Few long and short straight bacilli.
Dog B 2:				
Kidney	II.	0	Staphylococcus.	Spores and long straight bacilli.
Liver	II.	0	Staphylococcus. Mycoides.	Long straight bacilli like mycoides and spores.
Dog B 3:				
Kidney	III.	0	0	Few sporelike bodies and long straight bacilli.
Liver	III.	0	Staphylococcus. Spores. Long straight bacilli.	0

Gas produced in all broth-tubes of Series X. except Liver III.
Growths obtained at end of seventh day.

SET VI.

SERIES XI.—*Rabbits Killed Full Fed.*

Organs removed aseptically; thrown into bichloride of mercury solution 1:1000 *for one hour; passed into sterile water for one hour, and then washed with hot sterile water.*

Organs preserved dry in Petri dishes; preserved in agar plates and in broth-tubes.

Rabbit E 1:

Kidney	I.	Dry	=	Short straight bacilli. (Colon?)
Kidney	I.	Broth	=	Short straight bacilli. (Colon?) Large bacilli. (Gas produced.)
Liver	I.	Dry	=	Colon.
Liver	I.	Agar	=	Colon.
Liver	I.	Broth	=	Colon. (Gas produced.)

Rabbit E 2:

Kidney	II.	Dry	=	0
Kidney	II.	Broth	=	0 (Gas produced.)
Liver	II.	Dry	=	0
Liver	II.	Broth	=	0 (Gas produced.)
Liver	II.	Agar	=	0

Rabbit E 3:

Kidney	III.	Dry	=	Colon and heavy thick bacilli.
Kidney	III.	Broth	=	Colon and heavy thick bacilli. (Gas produced.)
Liver	III.	Agar	=	0
Liver	III.	Dry	=	0
Liver	III.	Broth	=	Broad straight bacilli. (Gas produced.)

Rabbit E 4:

Kidney	IV.	Broth	=	Staphylococci and bacilli. (Gas produced.)
Kidney	IV.	Agar	=	Staphylococci.
Liver	IV.	Agar	=	Surface. Mesentericus. Long bacilli-like subtilis. Organ. Mesentericus. Colon-like bacilli.
Liver	IV.	Broth	=	Short and long straight bacilli. (Gas produced.)
Liver	IV.	Agar	=	Long and short thick bacilli.

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TOTALS. ANIMALS KILLED.

SET I.

SERIES I. (Rabbits fasting.)		SERIES II. (Rabbits full fed.)	
Kidney	I. +	Kidney	I. +
Liver	I. +	Liver	I. 0
Kidney	II. +	Kidney	II. +
Liver	II. 0	Liver	II. 0
Kidney	III. 0	Kidney	III. +
Liver	III. +	Liver	III. +

Number of cultures	12
Number of growths on cultures	8
Percentage	$66\frac{2}{3}$
Number of organs	12
Number bacteria-containing	8
Percentage	$66\frac{2}{3}$

SET II.

SERIES III. (Rabbits fasting.)		SERIES IV. (Rabbits full fed.)			
		Agar.		Broth.	
Kidney	I. +	Kidney	I. +	Kidney	I. +
Liver	I. +	Liver	I. +	Liver	I. +
Kidney	II. +	Kidney	II. 0	Kidney	II. +
Liver	II. +	Liver	II. +	Liver	II. +
Kidney	III. +	Kidney	III. 0	Kidney	III. +
Liver	III. +	Liver	III. 0	Liver	III. +

Number of cultures	18
Number of growths on cultures	15
Percentage	83.3
Number of organs cultivated	15
Number of bacteria-containing	13
Percentage	86.2

SET III.

SERIES V. Guinea-pig (full fed).				SERIES VI. Guinea-pig (fasting).			
Agar.		Broth.		Agar.		Broth.	
Kidney	I. +	Kidney	I. +	Kidney	I. 0	Kidney	I. 0
Liver	I. +	Liver	I. +	Liver	I. +	Liver	I. +
Kidney	II. 0	Kidney	II. +	Kidney	II. +	Kidney	II. +
Liver	II. +	Liver	II. +	Liver	II. +	Liver	II. +
Kidney	III. +	Kidney	III. +	Kidney	III. 0	Kidney	III. +
Liver	III. +	Liver	III. 0	Liver	III. 0	Liver	III. +

Number of cultures	24
Number of growths	18
Percentage	75.0
Number of organs	18
Number of bacteria-containing	14
Percentage	77.7


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Set IV.

Series VII. (Cats fasting.)

Agar.		Broth.	
Kidney	I. +	Kidney	I. +
Liver	I. +	Liver	I. +
Kidney	II. +	Kidney	II. 0
Liver	II. +	Liver	II. +
Kidney	III. 0	Kidney	III. 0
Liver	III. +	Liver	III. +

Number of cultures 24
Number of growths 20
Percentage 83.3

Number of organs 18
Number of bacteria-containing 14
Percentage 77.7

Series VIII. (Cats full fed.)

Agar.		Broth.	
Kidney	I. +	Kidney	I. +
Liver	I. +	Liver	I. +
Kidney	II. +	Kidney	II. 0
Liver	II. +	Liver	II. +
Kidney	III. +	Kidney	III. +
Liver	III. +	Liver	III. +

Set V.

Series IX. (Dogs full fed.)

Agar.		Broth.	
Kidney	I. 0	Kidney	I. +
Liver	I. +	Liver	I. +
Kidney	II. +	Kidney	II. +
Liver	II. +	Liver	II. +
Kidney	III. +	Kidney	III. +
Liver	III. +	Liver	III. +

Number of cultures 24
Number of growths 21
Percentage 87.5

Number of organs cultivated 18
Number containing bacteria 16
Percentage 88.8

Series X. (Dogs fasting.)

Agar.		Broth.	
Kidney	I. +	Kidney	I. +
Liver	I. +	Liver	I. +
Kidney	II. +	Kidney	II. +
Liver	II. +	Liver	II. +
Kidney	III. 0	Kidney	III. +
Liver	III. +	Liver	III. 0

Set VI.

Series XI. (Rabbits full fed.)

Kidney I. Dry +	Kidney II. Dry 0	Kidney III. Dry +	Kidney IV. Broth +
Kidney I. Broth +	Kidney II. Broth 0	Kidney III. Broth +	Kidney IV. Agar +
Liver I. Broth +	Liver II. Dry 0	Liver III. Agar 0	Liver IV. Agar +
Liver I. Dry +	Liver II. Broth 0	Liver III. Dry 0	Liver IV. Broth +
Liver I. Agar +	Liver II. Agar 0	Liver III. Broth +	Liver IV. Agar +

Number of cultures 20
Number of growths 13
Percentage 65.0

Number of organs cultivated 12
Number of bacteria-containing 9
Percentage 75.0

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Summary.

Total number of animals	34
" number of organs cultivated	93
" number of cultures	122
" number of growths on cultures	95
Percentage	77.8
Number of organs containing bacteria	75
Percentage	80.6

Autopsy Cultures. Royal Victoria Hospital.

II., 1900. Male, 45. Plastic peritonitis with pneumothorax.

Eight hours after death.

Kidney,	Colon.	
Liver,	Colon.	
Spleen,	Staphylococcus.	

III., 1900. Female, 42. Chronic interstitial nephritis and uræmia.

Twenty-five hours after death.

Liver,	Colon.	
Spleen,	Colon and paracolon.	

VI., 1900. Male, 64. Chronic phthisis.

Sixteen hours after death.

Kidney,	Colon and staphylococcus.	Gas produced (Cultures).
Liver,	Colon.	Gas produced (Cultures).
Spleen,	Staphylococcus.	
	Paracolon.	No gas produced.
	Long putrefaction (2) forms.	(Cultures.)

VIII., 1900. Female, 10. Tuberculous meningitis and miliary tuberculosis.

Sixteen hours after death.

Kidney,	Staphylococcus.	
Liver,	Staphylococcus.	
Spleen,	Staphylococcus.	

IX., 1900. Male, 58. Mitral stenosis and incompetency.

Twenty-four hours after death.

Kidney,	Staphylococcus.	Gas produced.
	Colon.	
	Long thick bacilli.	
Liver,	Colon.	
	Long forms.	Gas produced.
	Broad forms.	
Spleen,	Colon.	Gas produced.

X., 1900. Female 30. Cholecystenterostomy. Death from exhaustion; no peritonitis.

Eight hours after death.

Kidney,	Staphylococci.	
	Mixture of bacilli which did not grow.	Gas produced.
	Colon.	

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Liver,	Staphylococci.	
	Mixture of bacilli.	Gas produced.
	Colon.	
Spleen,	Staphylococci.	Gas produced.
	Mixture of bacilli.	

XI., 1900. Female, 18. Cerebral abscess following otitis media.

Sixteen hours after death.

Kidney,	Staphylococcus.	Gas produced.
	Mesentericus fuscus.	
Liver,	Staphylococcus.	Gas produced.
	Mesentericus fuscus (vulgatus).	
Spleen,	Staphylococcus.	Gas produced.
	Long narrow bacilli.	

XII., 1900. Female, 43. Alcoholic neuritis.

Six hours after death.

Kidney,	Staphylococcus	No gas produced.
	Mesentericus fuscus.	
Liver,	Staphylococcus.	Much gas produced.
	Colon.	
	Mesentericus fuscus.	
Spleen,	Staphylococcus.	
	Colon.	Much gas.
	Mesentericus vulgatus.	

XIII., 1900 Female, 65. General peritonitis following stricture and ulceration of rectum.

Eight hours post-mortem.

Kidney,	Staphylococcus.	Gas produced.
	Mixture of bacilli.	
Liver,	Mesentericus fuscus.	Gas produced.
	Mixture of bacilli.	
Spleen,	Mesentericus fuscus.	Gas produced.

Anaërobic Cultures. Royal Victoria Hospital.

X., 1900. Eight hours after death.

Kidney,	Staphylococcus.
Liver,	Staphylococcus.
Spleen,	Paracolon.

XI., 1900. Sixteen hours after death.

Liver,	Colon.
Liver,	Staphylococcus.
	Long bacilli.
Spleen,	Staphylococcus.
	Long bacilli.

XII., 1900. Six hours after death.

Kidney,	Staphylococcus.
Liver,	Staphylococcus.
Spleen,	Colon.

XIII., 1900. Eight hours after death.

Kidney,	Paracolon.
	Mixture of bacilli.
	Long narrow bacilli without visible growth.
Spleen,	Staphylococcus.



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AUTOPSY. ROYAL VICTORIA HOSPITAL.

II.—1900	Kidney +	XI.—1900	Kidney +
	Liver +		Liver +
	Spleen +		Spleen +
III.—1900	Kidney ?	XII.—1900	Kidney +
	Liver +		Liver +
	Spleen +		Spleen +
VI.—1900	Kidney +	XIII.—1900	Kidney +
	Liver +		Liver +
	Spleen +		Spleen +

ANAEROBES.

VIII.—1900	Kidney +	X.—1900	Kidney +
	Liver +		Liver +
	Spleen +		Spleen +
IX.—1900	Kidney +	XI.—1900	Kidney +
	Liver +		Liver +
	Spleen +		Spleen +
X.—1900	Kidney +	XII.—1900	Kidney +
	Liver +		Liver +
	Spleen +		Spleen +
		XIII.—1900	Kidney +
			Liver +
			Spleen +

Total: 26 organs cultivated, all contain bacteria.

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Staining with Brazilin.

By

Sydney J. Hickson, F.R.S.,

Beyer Professor of Zoology in the Owens College, Manchester.

Brazilin is extracted from the wood of Cæsalpinia echinata, growing in South America. Formerly, under the name Brazil-wood, it was used commercially as a dye; but of recent years it has been superseded by other colouring substances, and practically driven out of the market.

Chemically it has considerable resemblance to hæmatoxylin, which is also extracted from the wood of a tree belonging to the order Leguminosæ, but brazilin ($C_{16}H_{14}O_5$) differs from hæmatoxylin ($C_{16}H_{14}O_6$) in having one hydroxyl less. Further information concerning the chemistry of brazilin may be found in the papers by Professor Perkin and Dr. Gilbody published in the 'Proceedings of the Chemical Society' during the past four or five years.

Brazilin has been tried as a stain for animal tissues by Flechsig and Breglia, but apparently the results were not very satisfactory. Their methods, though mentioned in the third edition of Lee's 'Microtomist's Vade-Mecum,' are omitted from the fourth edition.

Brazilin is mentioned as a chromatin stain in Rawitz's 'Leitfaden für histologische Untersuchungen,' p. 98, but it is not mentioned in the elaborate 'Tabellen zum Gebrauch bei mikroscopischen Arbeiten,' by W. Behrens, published in 1898, nor in Böhm and Oppel's 'Taschenbuch der mikroscopischen Technik,' published in 1896.

My first experiment was to attempt to use brazilin instead of hæmatoxylin in the method suggested by Heidenhain,

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were equally well defined in the two preparations; but the iron-brazilin showed an advantage in that the spermatozoa were triple stained, the head, middle piece, and tail being clearly of different colours.

A preparation of the sciatic nerve of a dog stained in brazilin exhibits the neurokeratin remarkably clearly, but the axis-cylinder is not well stained.

Our method has great advantages in showing the blood-supply of an organ, the red blood-corpuscles being stained very deeply. Preparations of ovary of a cat and kidney of a dog exhibit the capillary plexus excellently.

It is, however, principally in cytological work I think that the new method will be valuable, as we have found after many experiments, in our investigations on the very delicate nuclear structures of the Suctorian Dendrocometes, that it gives us by far the best general results.

It is obvious that a good deal more must be done before an opinion can be expressed as to the general value of this method, but I have thought it right to bring it before the notice of workers in various branches of microscopic science at the present time, because I feel that it is a method well worthy of further trial.

In conclusion I may say that the somewhat severe test of exposing slides to the action of direct sunlight for several months has not indicated any appreciable fading effect.

I am indebted to Professor W. H. Perkin, jun., F.R.S., for much valuable advice and assistance in working out this method.

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DESCRIPTION DU MATÉRIEL D'UNE PETITE INSTALLATION SCIENTIFIQUE.

1re Partie.

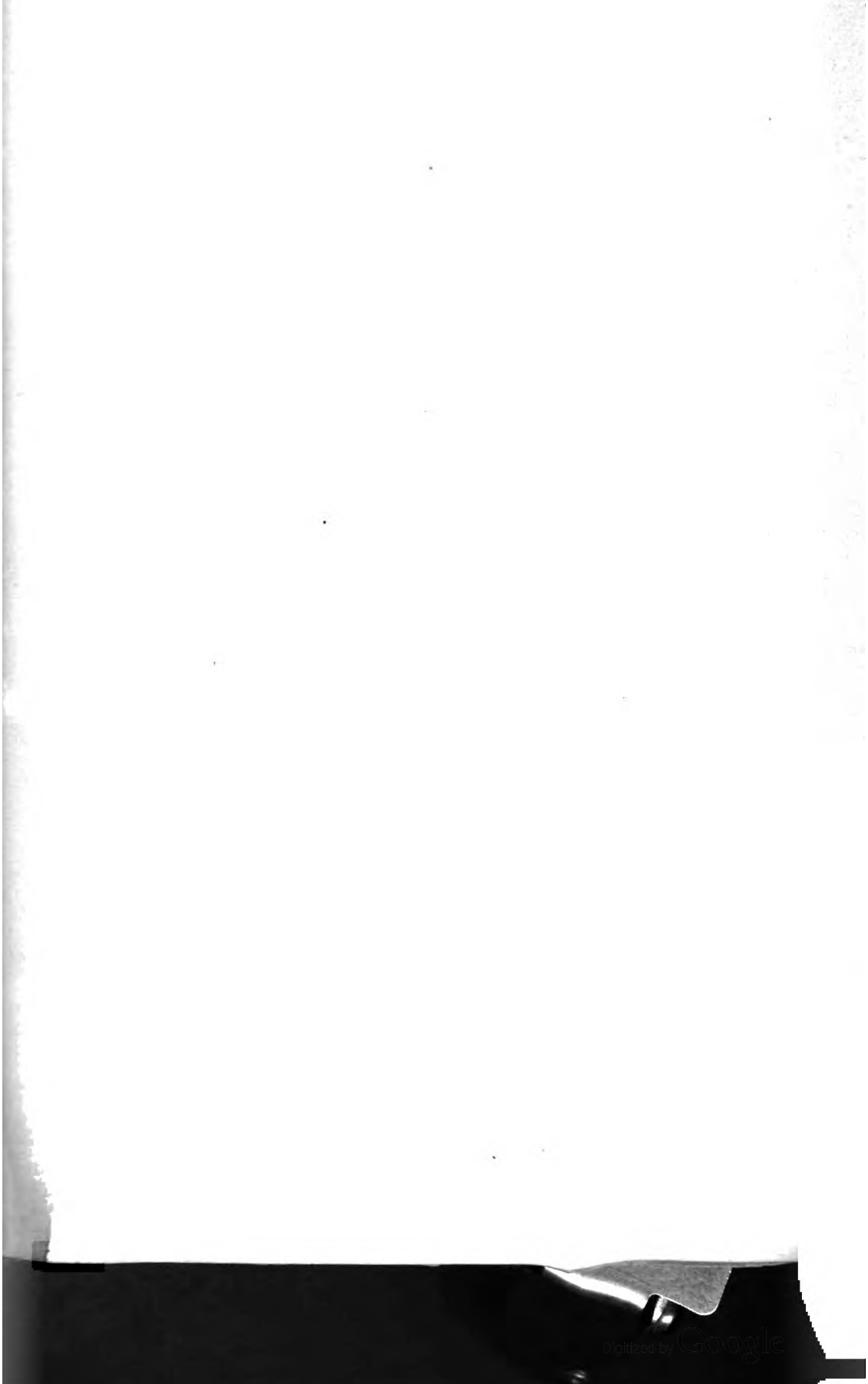
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DESCRIPTION

du

MATÉRIEL

d'une

PETITE INSTALLATION SCIENTIFIQUE

1re Partie

LIMOGES
IMPRIMERIE-LIBRAIRIE DUCOURTIEUX ET GOUT
7, RUE DES ARÈNES, 7
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DESCRIPTION DU MATÉRIEL

d'une

PETITE INSTALLATION SCIENTIFIQUE

1re Partie

Toute installation, quelque modeste qu'elle soit, doit faire l'objet, avant son exécution, d'un projet étudié dans ses moindres détails. Il est toujours utile, pour l'établissement de semblables projets, d'avoir quelques documents sur les installations similaires qui ont pu être réalisées précédemment.

C'est pour contribuer à fournir des documents de ce genre que je donne les dimensions et les dispositifs que j'ai adoptés pour le matériel d'une petite installation scientifique que j'ai réalisée à Beauvais.

Cette petite installation a eu pour but de classer :

1° Une Collection paléontologique d'Invertébrés ;

2° Un petit Matériel scientifique (produits chimiques ; ustensiles de chimie ; appareils de photographie ; instruments de dissection et de micrographie ; outillage d'atelier ; boîtes de catalogues sur fiches ; fournitures diverses) ;

3° Une petite Bibliothèque scientifique ;

4° Une série de Documents contenus dans des chemises ou dossiers en carton.

La description de l'installation de la Collection paléontologique fait, seule, l'objet de la présente notice.

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INSTALLATION
d'une
COLLECTION PALÉONTOLOGIQUE
D'INVERTÉBRÉS

Les échantillons de cette collection sont collés sur des planchettes. Exceptionnellement, s'ils sont rares et fragiles, ils sont logés soit dans des tubes en verre collés sur des planchettes, soit dans des cuvettes en carton. Ainsi disposés, et soigneusement étiquetés, les échantillons sont rangés dans des meubles à tiroirs.

MEUBLES A TIROIRS POUR LE RANGEMENT DES ÉCHANTILLONS

Les meubles à tiroirs qui servent au rangement des échantillons sont construits en chêne et en sapin.

Les bois employés sont indiqués dans le tableau suivant :

DÉSIGNATION DES BOIS		ÉPAISSEURS du BOIS BRUT	ÉPAISSEURS maxima DU BOIS RABOTÉ
Chêne.	Feuillets sur quartier....	11 1/4 millim.	10 millimètres
		13 1/2 —	12 —
		16 —	14 —
	Planches sur quartier....	27 —	25 —
		34 —	32 —
		41 —	39 —
Sapin.	Feuillets de madrier 4 traits	13 —	11 —
	Planches ordinaires.....	25 —	22 —
	Planches dites cinq quarts.	33 —	30 —

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Tiroirs

Les tiroirs sont de deux grandeurs.

Ceux d'une même grandeur sont, tous, parfaitement interchangeables.

Leur dimension transversale et leur dimension sagittale ont été prises aussi grandes qu'on a pu le faire sans en rendre le maniement trop difficile.

Environ 95 pour 100 des échantillons de la collection ont, y compris la planchette de 8 millimètres d'épaisseur sur laquelle ils sont collés, une hauteur verticale inférieure à 75 millimètres. Ces échantillons, que l'on peut appeler *petits échantillons,* se logent dans les tiroirs de la plus petite des deux grandeurs.

Les plus grands tiroirs servent à loger les échantillons qui, avec la planchette sur laquelle ils sont collés, ont une hauteur comprise entre 75 et 100 millimètres et que l'on peut appeler *échantillons moyens.*

Les *gros échantillons,* ayant, avec leur support, plus de 100 millimètres de hauteur, ne peuvent guère se loger commodément dans des tiroirs.

Tiroirs pour les petits échantillons

Les tiroirs destinés à loger les petits échantillons sont représentés sur les planches 1 à 7.

Ils ont intérieurement :

Dimension transversale.....	673	millimètres.
— sagittale.....	546	—
— verticale.....	76	—

Avec ces dimensions, les tiroirs pour les petits échantillons sont suffisamment maniables pour pouvoir être placés, couramment, dans des cases situées à moins de deux mètres au-dessus du sol et, exceptionnellement, à une hauteur un peu plus grande.

Ces tiroirs sont entièrement en chêne.

Ils sont formés (pl.4) d'un corps de tiroir, d'une devanture rapportée, fixée au moyen de vis, et, entre ces deux parties, d'une plaque de feutre.

Pour faciliter l'entrée des tiroirs dans leurs cases, l'angle supérieur de l'extrémité arrière des côtés est abattu en pan coupé, et le derrière du tiroir a, en hauteur, 10 millimètres de moins que les côtés.

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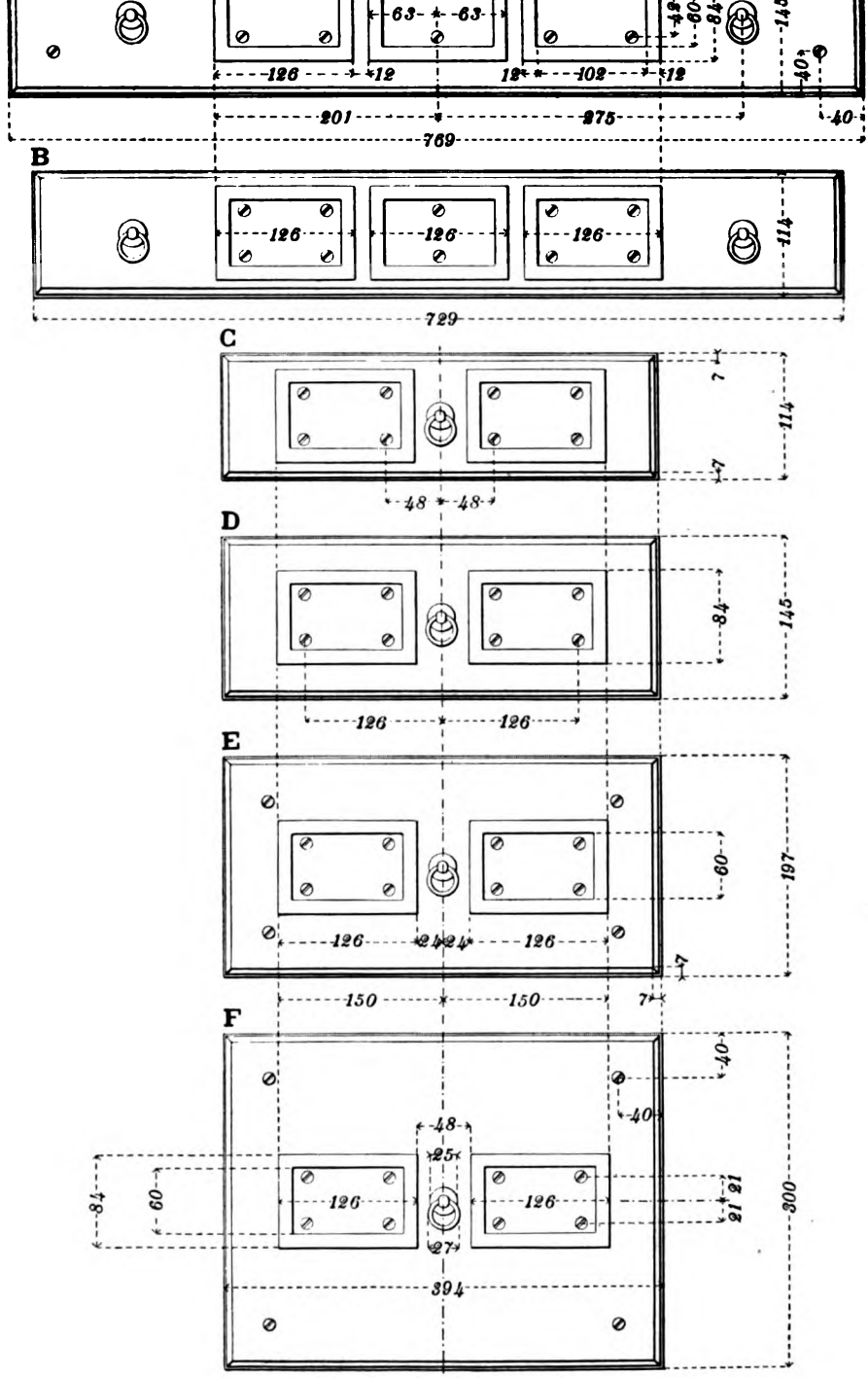


Fig. 1. — Façades des tiroirs. Ech. 0,15.

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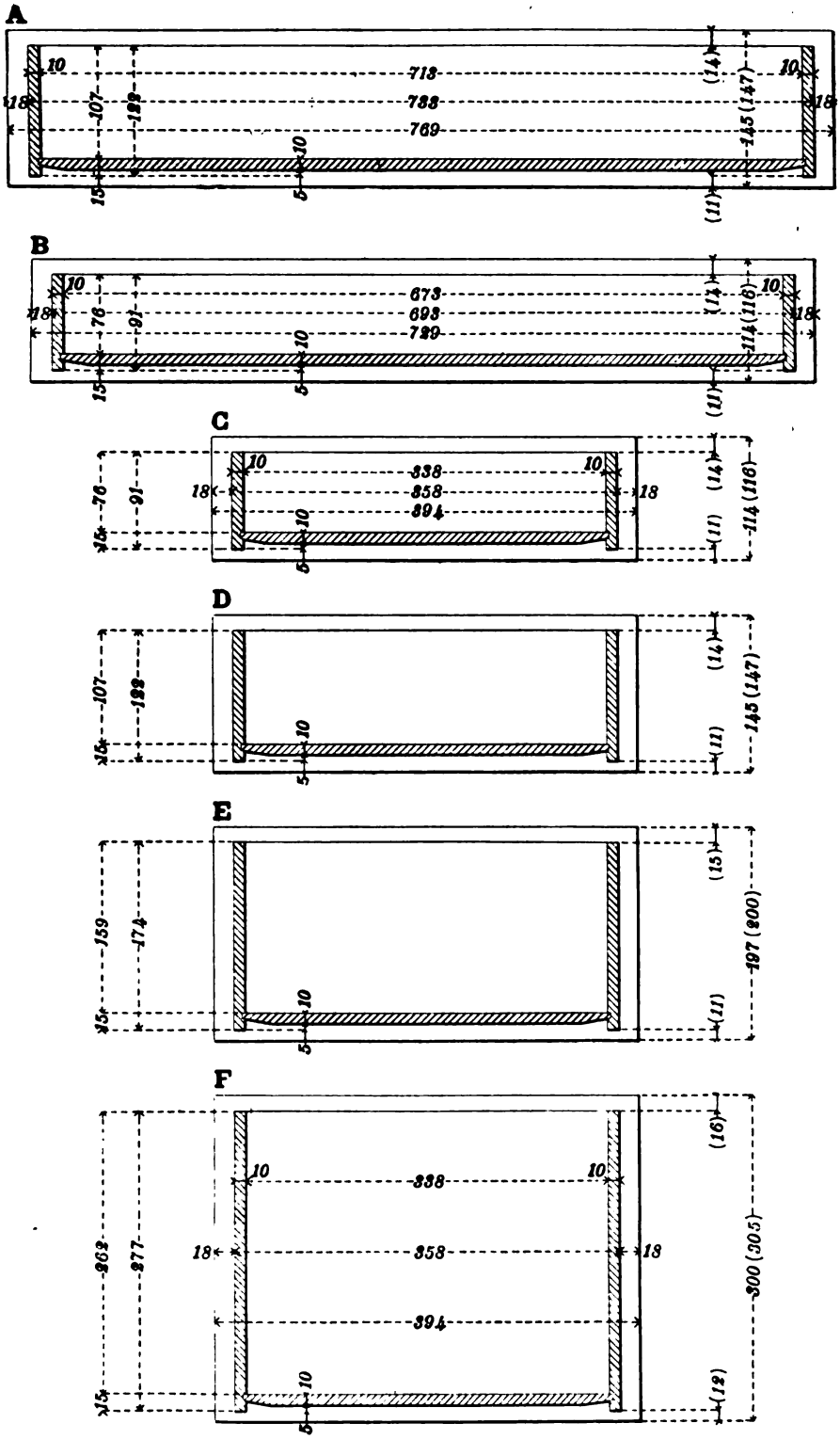


Fig. 2. — Coupe transversale des tiroirs. Ech. 0,15.

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Le fond horizontal du tiroir est soutenu :

En avant, par deux vis à tête fraisée (n° 17 × 20^{mm}) dont l'emplacement est indiqué, par deux petites croix, sur la planche 2.

En arrière (pl. 2 et 4), par une équerre en cuivre dont la branche horizontale est logée dans une entaille creusée sur la face inférieure du fond, tandis que sa branche supérieure, solidement maintenue par deux vis, est soutenue par une mouse logée dans une petite entaille.

Sur les côtés (pl. 3), par les rainures dans lesquelles pénètrent les fonds.

Pour ne pas produire, à l'arrière des tiroirs, partie qui est exposée à des chocs, un affaiblissement trop prononcé des côtés au droit des rainures, ces dernières sont poussées obliquement, de manière à avoir 6 millimètres de profondeur à l'avant et seulement 2 millimètres de profondeur à l'arrière. Il en résulte que le fond doit être découpé non pas tout à fait d'équerre, mais, un peu, en forme de trapèze. Cette disposition donne beaucoup de solidité aux côtés des tiroirs.

On fera bien, si l'on ne dispose pas de bois parfaitement secs, d'établir les tiroirs neufs avec des fonds faisant un peu saillie vers l'arrière. Dans ce cas, l'équerre en cuivre, au lieu d'être appliquée directement sur le corps du tiroir, en sera séparée par une petite cale d'épaisseur égale à la saillie provisoire du fond. On supprimera cette cale quand le bois sera à peu près complètement desséché. Avec des fonds ayant, comme c'est ici le cas, environ 0^{m}56, dans le sens transversal par rapport aux fibres du bois, le rétrécissement peut, au bout d'une dizaine d'années, atteindre 10 millimètres.

Les façades rapportées sont moulurées sur leur pourtour. Avec le feutre contre lequel elles sont appliquées, elles forment, sur la devanture du casier, un recouvrement peu pénétrable à la poussière. Pour être tout à fait efficace, ce recouvrement doit avoir 10 millimètres, ce qui conduit à donner aux façades rapportées 114 millimètres de largeur. Pour être sûr de conserver cette largeur, il faut employer du bois bien sec. Si le bois dont on dispose ne répond pas à cette condition, il faut préparer les devantures neuves avec un léger excédent de largeur. C'est ce qui a été fait pour les devantures des tiroirs représentés sur la figure 2. Ces devantures ont, en effet, été exécutées avec les largeurs et les saillies de recouvrement, un peu forcées, qui sont indiquées entre parenthèses, sur cette figure et dans le tableau de la page 34.

Pour protéger efficacement contre la pénétration de la poussière, le feutre employé doit être épais et mou.

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La sorte que j'ai adoptée a, mesurée, sans compression, avec un pied à coulisse de mécanicien, environ 12 millimètres d'épaisseur. A l'usage, par suite de la compression, cette épaisseur se réduit à 10 ou 11 millimètres.

Pour mettre ce feutre, autant que possible, à l'abri des attaques des insectes, il est indispensable de l'imbiber d'un liquide conservateur. Celui que j'emploie a la composition suivante :

Eau de pluie.....	950	grammes.
Bichlorure de mercure ordinaire.....	33	—
Chlorure d'ammonium sublimé blanc...	17	—

On obtient, rapidement, la dissolution de ces deux sels en les plaçant dans un nouet de toile que l'on maintient, suspendu, dans la partie supérieure de l'eau destinée à les dissoudre.

L'addition de chlorure d'ammonium a été suggérée par le chimiste Cloez pour éviter la transformation du bichlorure de mercure ou chlorure mercurique (sublimé corrosif), qui est si toxique, en chlorure mercureux (calomel) qui paraît être à peu près inoffensif pour les insectes.

Lorsqu'elles seront définitivement posées et ajustées, les garnitures de feutre auront exactement, comme les devantures rapportées qui les recouvrent, 729 sur 114 milimètres. Mais il faut tenir compte, pour le découpage des morceaux à imbiber, de ce que l'imbibition et la dessication produisent un certain retrait, et de ce qu'il faut laisser un excédent de dimension pour permettre de couper, bien nettement, le feutre après la pose. Pour être certain de pouvoir ajuster facilement la garniture de feutre à cette dimension de 0^m729, il faut que les morceaux, découpés avant l'imbition, aient au moins 0^m80. Les dimensions courantes des pièces de feutre que j'emploie étant de 1^m40 sur, environ, 20 mètres, on peut les découper en morceaux ayant 0^m80 sur 1^m40 que l'on plonge, après les avoir mis en rouleaux de 80 centimètres de longueur sur 15 à 20 centimètres de diamètre, dans le récipient rempli du liquide conservateur dont on veut les imprégner.

Les rouleaux de feutre restent plongés dans ce liquide pendant quarante-huit heures, puis, après avoir été bien égouttés, ce qui est important pour que le feutre ne devienne pas hygrométrique, étalés à plat sur un plancher où on les laisse sécher.

Les premières garnitures de feutre que j'ai employées, sans les imbiber d'un liquide conservateur, présentaient, au bout de cinq ou six années, un certain nombre de perforations, surtout au niveau de la partie inférieure des côtés des tiroirs, endroit qui constitue un point d'attaque de prédilection.

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Quant aux garnitures imbibées du liquide dont la composition est indiquée ci-dessus, elles ne présentent, au bout de dix années, aucune perforation.

Chacune des façades rapportées est fixée, au-devant du corps du tiroir, par douze vis en fer à tête fraisée n° 22×32 millimètres. Dix de ces vis ont leur tête cachée sous les étiquettes des tiroirs. Pour obtenir un serrage énergique du feutre, il est indispensable que les trous, percés un peu juste, dans lesquels pénètrent les filets de ces vis, traversent de part en part le devant du corps du tiroir. Les deux autres vis sont placées, en sens inverse des précédentes, dans l'axe de chacune des platines des deux anneaux, et les trous qui reçoivent les filets de ces vis traversent, de part en part, la devanture rapportée.

Il n'est pas toujours nécessaire de poser toutes les douze vis de chaque façade, mais il faut, néanmoins, préparer les douze trous destinés à les recevoir. Au moment où l'on place définitivement la devanture rapportée sur la plaque de feutre, on pose, d'abord, dans l'intérieur de chacun des deux porte-étiquettes latéraux, les deux vis voisines du côté extérieur du porte-étiquette, et, ensuite, les deux vis qui correspondent aux deux anneaux. Quant aux six autres vis, on ne place que celles qui sont nécessaires pour dérondir ou dégauchir la devanture rapportée, et la forcer à être bien plane et bien parallèle à la devanture du casier, de manière que la plaque de feutre y soit bien appliquée et ne laisse aucun jour.

Lorsque le morceau de feutre est solidement maintenu par le serrage énergique des vis de la devanture rapportée, on l'ajuste, exactement, à la dimension de cette devanture en coupant, bien nettement, tout ce qui dépasse son pourtour. Cette opération se fait avec un fort tranchet ou avec un de ces instruments, employés pour couper le cuir, tels que la cornette à couper des bourreliers, ou le couteau, à lame demi-circulaire, à manche central, que l'on appelle couteau à pied.

Les dimensions des tiroirs pour les petits échantillons sont résumées dans le tableau qui se trouve, ci-après, page 34.

Afin de faciliter, à l'ouvrier qui construit les meubles, la vérification des dimensions principales, de celles, surtout, qui doivent être observées avec une exactitude suffisante pour assurer l'interchangeabilité parfaite des tiroirs, il est indispensable d'employer des gabarits indicateurs de ces dimensions.

L'un des meilleurs dispositifs à adopter, pour des gabarits de ce genre, est celui indiqué sur la figure 9 qui représente un gabarit destiné à la vérification des dimensions d'un casier. Les butées, qui

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servent, deux à deux, à déterminer une dimension donnée, sont séparées du corps du gabarit par un trait de scie qui permet de les ajuster, facilement, avec toute la précision voulue. On peut, en effet, non seulement raccourcir ces butées en les limant, mais on peut aussi, lorsque c'est nécessaire, les allonger en les martelant.

Tiroirs pour les échantillons moyens

Les tiroirs pour le classement des échantillons moyens sont construits sur le même plan que ceux pour les petits échantillons, mais ils sont notablement plus grands.

Ils ont intérieurement :

Dimension transversale.	713	millimètres.
— sagittale.....	607	—
— verticale.	107	—

Les devantures rapportées des tiroirs pour les échantillons moyens étant notablement plus grandes que celles des tiroirs pour les petits échantillons, il a été nécessaire d'ajouter (fig.1, p.6) à chacun des quatre angles de la devanture une vis en cuivre à tête ronde n° 22×35 millimètres, qui reste apparente.

Les dimensions de ces tiroirs pour les échantillons moyens sont résumées dans le tableau qui se trouve, ci-après, page 34.

Anneaux et porte-étiquettes des devantures des tiroirs

Les devantures des deux modèles de tiroirs qui viennent d'être décrits portent, chacune, deux anneaux de tirage et trois porte-étiquettes (fig.1, p.6, et pl.1,2,4,6).

Les anneaux montés sur une platine sont en laiton. Ils sont fixés aux tiroirs par trois vis en laiton, à tête fraisée, n° 16×10 millimètres.

Les dimensions de ces anneaux sont :

Diamètre extérieur de l'anneau...	27	millimètres.
— — de la platine.....	25	—
— — du bouton.....	10	—
— du fil de laiton.....	3	—
Epaisseur de la platine.....	3	—
Epaisseur totale de la platine et du bouton.	12	—

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Les porte-étiquettes sont en noyer choisi sans aubier. Ils sont fixés aux devantures par sept vis en cuivre à tête ronde, n° 13×13 millimètres.

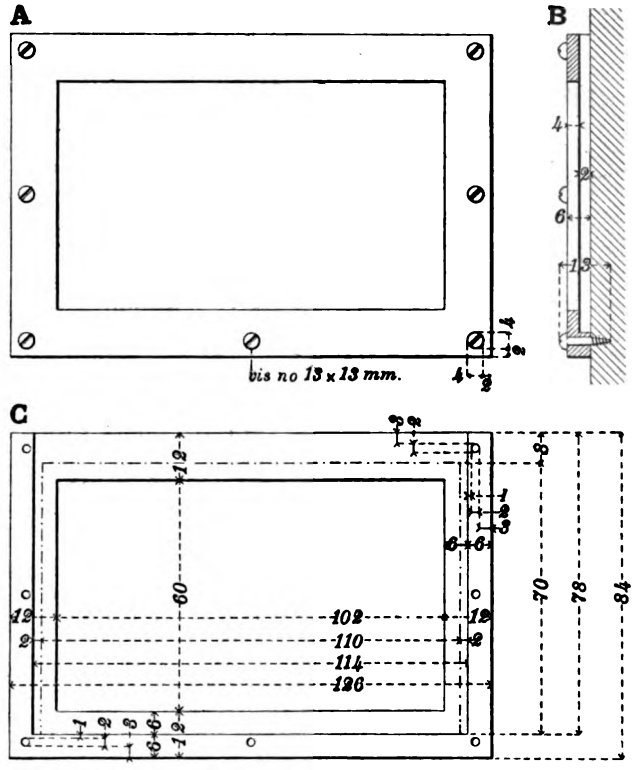


Fig. 3. — Porte-étiquettes des devantures des tiroirs. Les lignes formées de tirets alternant avec des points indiquent les dimensions des étiquettes en carte bristol. Ech. 0,5.

Les dimensions relatives à ces porte-étiquettes sont :

Dimension transversale	de l'évidement à jour.....	102	millimètres
	de l'étiquette en bristol.....	110	—
	dans feuillure.....	114	—
	extérieure.....	126	—
Dimension verticale...	de l'évidement.....	60	—
	de l'étiquette en bristol.....	70	—
	dans feuillure.....	78	—
	extérieure.....	84	—
Epaisseur.....	de la feuillure.....	2	—
	de la bordure de recouvrement	4	—
	extérieure.....	6	—

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Casiers des tiroirs pour les petits échantillons

Les tiroirs pour les petits échantillons sont groupés, par cinq, dans des casiers de petites dimensions que l'on peut juxtaposer et superposer de manière à en faire les éléments de tables-comptoirs ou de meubles de grande hauteur (planches 1 à 7). Grâce à ce dispositif, le démontage, le déménagement et le remontage des meubles est une opération relativement simple.

Ces casiers ont :

Dimension transversale extérieure		775	millimètres.
—	sagittale —	605	—
—	verticale —	650	—

Afin que, lors de leur juxtaposition, les casiers puissent être bien serrés les uns contre les autres et puissent former une façade bien droite, il est utile de leur donner une largeur extérieure un peu plus faible en arrière qu'en avant. Une différence de 2 millimètres est suffisante. La largeur extérieure du casier qui a 775 millimètres sur la façade, n'a, ainsi, que 773 millimètres en arrière.

Ces casiers sont construits partie en chêne, partie en sapin.

Le chêne est indiqué, sur les dessins, par des hachures assez serrées, le sapin, par des hachures plus espacées.

Les ouvertures des cases, mesurées sur la façade, laissent, pour faciliter l'entrée des tiroirs, un jeu de 4 millimètres en largeur et de 3 millimètres en hauteur.

A moins de construire les tiroirs et les casiers avec une précision qui se traduirait par un prix de revient relativement élevé, ce jeu serait, parfois, insuffisant si le coulisseau de droite d'une case était placé parallèlement au coulisseau de gauche.

Afin d'assurer, d'une façon absolue, l'interchangeabilité des tiroirs, les joues des coulisseaux (pl.2) sont entaillées de 6 millimètres et divergent de manière à présenter, en arrière, un écartement plus grand que leur écartement en avant. La différence d'écartement est égale à la somme des deux entailles de 6 millimètres, diminuée du rétrécissement de 2 millimètres que le casier présente extérieurement en arrière, ce qui fait 10 millimètres. L'écartement des joues des coulisseaux qui est, en avant, comme l'ouverture des cases, de 697 millimètres, est, ainsi, de 707 millimètres en arrière.

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Les coulisseaux et leurs joues (pl.2 et 4) sont taillés en biseau à leur extrémité voisine de la façade. Ce dispositif a pour but d'empêcher qu'il n'y ait, par suite de désaffleurements, des saillies sur lesquelles les tiroirs viendraient buter.

Il est également utile, pour que les tiroirs ne soient pas exposés à venir buter sur elle, que la traverse située en bas et en arrière des casiers (pl.4) n'arrive pas tout-à-fait au niveau des coulisseaux inférieurs. La différence est ici de 5 millimètres.

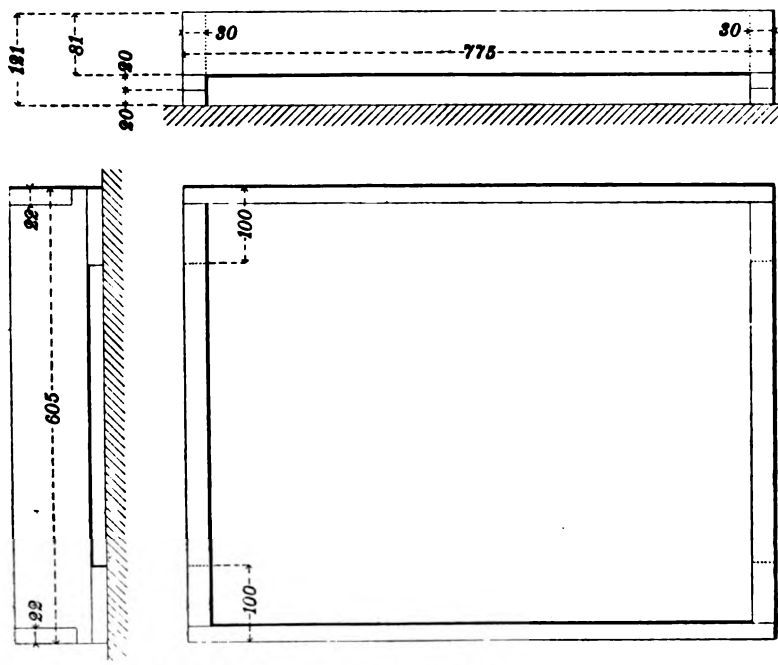


Fig. 4. — Support pour les casiers formant tables-comptoirs. Ech. 0,1.

Dans les derniers casiers que j'ai fait construire, les deux tringles verticales (pl.2) et les deux tringles horizontales (pl.4) qui sont rapportées, pour former joue de feuillure, et maintenir à dilatation libre les panneaux de derrière des casiers, sont faites en chêne, et ont 10 sur 60 millimètres d'équarissage, au lieu d'être en sapin, et de n'avoir que 10 sur 40 millimètres, comme l'indiquent les dessins.

Les dimensions principales de ces casiers sont résumées dans le tableau qui se trouve, ci-après, page 34.

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Casiers des tiroirs pour les échantillons moyens

Les casiers des tiroirs pour les échantillons moyens sont construits exactement sur le même plan que ceux des tiroirs pour les petits échantillons.

Ils ont la même hauteur, à savoir 650 millimètres, mais ils sont plus larges et plus profonds et n'ont que quatre cases.

Les dimensions de ces casiers se trouvent dans le tableau de la page 34.

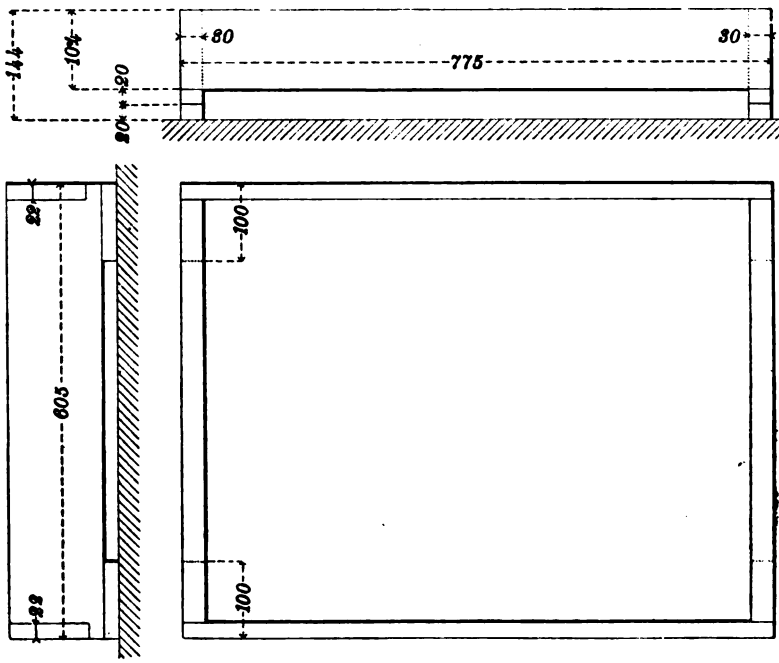


Fig. 5. — Support pour les grands meubles formés de quatre assises de casiers. Ech. 0,1.

Groupement des Casiers

Meubles pour les petits échantillons

Les casiers à tiroirs pour les petits échantillons sont destinés à être juxtaposés et superposés pour former des tables-comptoirs ou des meubles de plus grande hauteur (pl. 5, 6 et 7).

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TABLES-COMPTOIRS

Les tables-comptoirs (pl. 5, fig. 5 et 6) sont formées d'une assise de casiers juxtaposés. Chacun des casiers est posé sur un support (fig. 4, p. 14) formé de deux longuerines réunies par deux traverses. Les deux longuerines supportent efficacement les quatre montants du casier ainsi que ses traverses latérales inférieures. Elles reposent sur le sol par quatre cales que l'on peut amincir pour compenser les irrégularités de l'horizontalité du plancher.

Le pourtour de l'ensemble des supports reçoit une plinthe moulurée, de 128 millimètres de hauteur, qui recouvre, de quelques millimètres, les traverses inférieures des casiers (pl. 5).

Une solide table en chêne de 25 millimètres d'épaisseur, à bords arrondis, formée de planches collées mais non entourées d'emboîtures, est fixée sur l'assise de casiers. Le dessus de cette table est à $0^m 800$ du sol, hauteur qui permet d'étaler commodément, sous la main, les tiroirs et les échantillons.

GRANDS MEUBLES

Les grands meubles à tiroirs sont formés de plusieurs assises de casiers superposées au-dessus d'un support semblable à ceux employés pour les tables-comptoirs, mais plus haut (fig. 5, p. 15, pl. 5; fig. 7 et 8, pl. 6).

La hauteur la plus commode à donner à ces grands meubles est celle qui résulte de la superposition de trois assises de casiers, superposition qui donne des piles de quinze tiroirs. Dans ce cas, avec un socle de 150 millimètres, les anneaux de tirage des tiroirs placés tout en haut du meuble se trouvent à peu près à 2 mètres au-dessus du plancher, c'est-à-dire encore à portée de la main. On aura soin, lors de chaque remaniement de la collection, de laisser, dans chaque pile de quinze tiroirs, quelques tiroirs vides, pour permettre l'intercalation de nouveaux échantillons. C'est avec ce dispositif que mes premières installations ont été faites, et c'est celui que j'aurais conservé si j'avais disposé d'emplacements suffisamment vastes.

Pour l'installation de la collection, dans un local très restreint, il a fallu donner aux meubles une plus grande hauteur et les former par la superposition de quatre assises de casiers, ce qui donne des piles de vingt tiroirs.

Les cinq tiroirs logés dans le casier supérieur ne sont plus à la portée de la main et ne sont accessibles qu'avec l'emploi d'un escabeau d'environ $0^m 75$ de hauteur, formé de trois marches.

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Ce serait là un inconvénient assez sérieux, s'il fallait atteindre fréquemment les tiroirs de ce casier supérieur ; mais, en réalité, cela n'a lieu que d'une façon exceptionnelle, si on a soin de ne remplir, dans chaque pile de vingt tiroirs, que les quinze tiroirs inférieurs, et de conserver les cinq tiroirs supérieurs pour permettre les remaniements partiels, nécessités par l'intercalation des nouveaux échantillons. Dans ce cas, tous les tiroirs situés à portée de la main sont utilisés, et les tiroirs peu accessibles ne le sont que momentanément, jusqu'à ce qu'un remaniement plus important permette de les libérer à nouveau.

Cette disposition de grands meubles formés par la superposition de quatre assises de casiers est représentée sur les planches 5, 6 et 7.

Le soubassement mouluré a 150 millimètres de hauteur. Il recouvre de 6 millimètres la traverse du bas des casiers de l'assise inférieure.

De petites cales séparent les casiers superposés et compensent les petites différences qui existent dans leurs dimensions extérieures.

Les joints résultant de l'intercalation de ces petites cales sont, de même que les joints qui séparent les piles juxtaposées, masqués par une petite moulure de 18 millimètres de largeur.

Le couronnement a 200 millimètres de hauteur et recouvre de 6 millimètres la traverse du haut des casiers de l'assise supérieure.

Avec ce système de juxtaposition et de superposition, tous les panneaux de dessus, de dessous et de derrière des casiers paraissent inutiles, et il semble qu'il suffirait d'avoir seulement un certain nombre de panneaux de côté, pour former les flancs des meubles.

En réalité, tous les panneaux sont utiles, si l'on tient à se mettre, le plus possible, à l'abri de la poussière, parce qu'ils s'opposent, d'une façon très efficace, à la circulation de l'air dans l'intérieur des casiers.

Meubles pour les échantillons moyens

Beaucoup plus volumineux et beaucoup plus lourds que ceux des petits échantillons, les tiroirs des échantillons moyens ne peuvent être placés dans des meubles de grande hauteur. Ils sont assez maniables s'ils sont placés dans des tables-comptoirs de 0m800 de hauteur, formées, comme les tables-comptoirs des tiroirs pour les petits échantillons, d'une seule assise de casiers.

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Exemple d'installation d'une Salle

Les meubles qui viennent d'être décrits peuvent être disposés de façons très variées suivant la forme et les dimensions des locaux dont on dispose. Ils se prêtent à des installations satisfaisantes même dans des locaux présentant, comme la salle où j'ai dû loger ma collection, des irrégularités assez prononcées.

La planche 7 donne un exemple de l'une des meilleures dispositions qui puissent être adoptées dans un local de forme parfaitement régulière. On a supposé, dans cet exemple, qu'une salle de 9^m72 sur 11^m, traversée par un passage de 2^m05 est garnie de grands meubles, formés de quatre assises de casiers pour petits échantillons, alternant avec des tables-comptoirs formées, sans réserver de vides, de casiers semblables aux précédents. Une salle ainsi meublée contient mille tiroirs.

Si l'on fait une installation similaire, mais avec des meubles formés seulement de trois assises de casiers et avec des tables reposant sur des pieds et non sur des casiers, la même salle ne contient plus que six cents tiroirs.

Il est utile de laisser, entre les fenêtres et les tables-comptoirs, un passage ayant, au moins, 50 centimètres, ou mieux, si l'emplacement le permet, 60 à 75 centimètres de largeur.

Les grands meubles ne sont pas placés au contact immédiat des murs. Ils en sont séparés par un espace, d'environ 2 centimètres (pl.7), dans lequel on place, à l'alignement de la façade, une petite tringle en chêne ajustée de manière à racheter les irrégularités des murs et le faux équerre de la construction.

Dans l'exemple d'installation représenté par la planche 7, les tables-comptoirs sont formées comme les grands meubles de deux lignes de casiers accolées dos à dos, et ayant, ensemble, une largeur de 1^m210. Les passages entre les façades des casiers ont cette même largeur de 1^m210. Cette disposition, que j'ai eu l'occasion d'essayer dans une installation provisoire, est très commode pour le maniement des tiroirs.

Dans l'installation que j'ai réalisée pour ma collection, j'ai été obligé, pour gagner de la place, de réduire sensiblement ces deux dimensions.

Je n'ai pu former les tables-comptoirs que d'une seule ligne de casiers ayant transversalement 0^m605, avec tables de 0^m67 de largeur. Cette dimension est encore bien suffisante pour poser les tiroirs et étaler des séries d'échantillons.

Quant aux passages entre les comptoirs et les grands meubles, j'ai du également les réduire, et j'ai constaté que cela peut être fait, sans trop d'inconvénients, à la condition, toutefois, de ne pas leur donner une largeur inférieure à 1 mètre.

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Matériel pour les échantillons

Planchettes

Les planchettes sur lesquelles sont collés les échantillons sont en bois bien sec (aulne, bouleau, peuplier) de 8 millimètres d'épaisseur, découpées bien d'équerre.

Elles sont débitées à la scie fine, et poncées sur une face pour faire disparaître le trait de scie. Ce ponçage n'est pas nécessaire si l'on emploie du peuplier tranché, parce que la surface de cette sorte de bois est suffisamment unie.

Près de 75 pour cent des échantillons de la collection en question ne dépassent pas 30 millimètres de largeur.

Pour ce motif, j'ai pris cette dimension comme point de départ de la série de dimensions transversales des planchettes qui est, ainsi, la suivante :

30; 60; 90; 120; 150 mm, etc.

Exceptionnellement, lorsque cela est utile pour obtenir une notable économie de place, j'emploie, de plus, les dimensions transversales :

15; 45; 75 et 105 mm.

Dans le sens sagittal, les dimensions des planchettes vont de 5 en 5 millimètres.

Les planchettes sont recouvertes, sur le dessus et sur les côtés, avec le papier bleu pâte dont un échantillon se trouve p. 24, en regard de la figure 6.

Ce papier est d'une teinte assez solide, parce que sa matière colorante (bleu de Prusse) reste assez fixe sous l'action de la lumière, et que la pâte employée pour sa fabrication ne contient pas de bois, matière qui jaunit assez rapidement et verdit les bleus les plus fixes. Des morceaux de ce papier, exposés pendant deux mois (mai et juin) dans des vitrines servant à faire blanchir, sous l'action du soleil, des objets manufacturés en os, n'ont subi qu'un léger changement de couleur.

Le dos de chaque planchette est recouvert de papier blanc pouvant servir à écrire des annotations.

Pour éviter une diminution de leurs dimensions et le décollement du papier qui les recouvre, les planchettes doivent être découpées dans du bois bien sec. De plus, pour compenser les

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petites irrégularités du sciage, le défaut d'équerre et l'augmentation de dimension due au recouvrage, il faut découper les planchettes avec un demi-millimètre de moins, en longueur et en largeur, que les dimensions indiquées ci-dessus. Avec ces précautions, les planchettes terminées ont des dimensions suffisamment exactes pour pouvoir être rangées bien régulièrement.

Une très bonne ouvrière, bien outillée, peut garnir de papier bleu et blanc, collés à la colle forte, en travail très soigné, quatre cents planchettes de dimensions moyennes en une journée de huit heures, soit cinquante à l'heure.

Une étiquette en papier blanc, pur chiffon, est collée au bas de chaque planchette (fig.6, p. 24).

Les dimensions et le nombre de lignes d'écriture adoptés pour ces étiquettes sont indiqués ci-dessous :

Largeur de la planchette.... millim.	15	30	45	60	75	90	105	120
Dimensions correspondantes de l'étiquette.... millim.	12×20	27×14	42×10	57×8	72×8	86×6	101×6	116×6
Nombre de lignes d'écriture sur l'étiquette	9	6	4	3	3	2	2	2

Le plus souvent, je ne mets, sur chaque planchette, qu'un seul échantillon. Cela facilite les intercalations, les remaniements, et, surtout, l'élimination des échantillons défectueux dès qu'on peut les remplacer par des échantillons meilleurs.

Toutefois, les individus très petits, les espèces très communes, les séries provenant d'un même gisement, les échantillons médiocres qui sont certainement destinés à être bientôt éliminés, doivent, de préférence, être groupés sur une même planchette.

Tubes

Les tubes, employés pour loger les échantillons rares et fragiles, sont faits en verre mince, de très belle qualité, aussi peu striés que possible. Ils sont à fonds plats, à bords coupés droits sans évasement, et bouchés avec un bouchon en liège enfoncé du tiers de sa longueur.

Les dimensions adoptées pour les tubes les plus usuels sont indiquées sur le tableau suivant :

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DIMENSIONS extérieures DES TUBES		ÉPAISSEUR du VERRE	LONGUEUR totale du BOUCHON
Diamètre	Longueur		
10 millimètres	20 millimètres	$^{9}/_{10}$ de millim.	9 millimètres
10 —	30 —	$^{9}/_{10}$ —	9 —
10 —	40 —	$^{9}/_{10}$ —	9 —
15 —	30 —	$^{10}/_{10}$ —	10 —
15 —	40 —	$^{10}/_{10}$ —	10 —
15 —	50 —	$^{10}/_{10}$ —	10 —
20 —	40 —	$^{11}/_{10}$ —	11 —
20 —	50 —	$^{11}/_{10}$ —	11 —
20 —	60 —	$^{11}/_{10}$ —	11 —
25 —	50 —	$^{12}/_{10}$ —	12 —
25 —	60 —	$^{12}/_{10}$ —	12 —
25 —	70 —	$^{12}/_{10}$ —	12 —
30 —	60 —	$^{13}/_{10}$ —	13 —
30 —	70 —	$^{13}/_{10}$ —	13 —
30 —	80 —	$^{13}/_{10}$ —	13 —

Cuvettes

Les cuvettes, destinées à recevoir les échantillons, que, pour une raison quelconque, l'on ne veut pas coller sur des planchettes, sont faites en carte de Lyon fine, format raisin simple, pesant 20 kilogrammes les cent feuilles, recouverte, sur les deux faces, avec le papier bleu qui sert à recouvrir les planchettes. L'épaisseur de cette carte est, après un fort glaçage, d'environ $\frac{7}{10}$ de millimètre.

Au moyen d'un petit gabarit, sur lequel se trouvent dessinés les tracés de toutes les tailles usuelles, l'ouvrière chargée de faire ces cuvettes peut les pointer et les découper rapidement sur la mesure de l'échantillon qui lui est donné.

Les dimensions extérieures horizontales de ces cuvettes sont,

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comme pour les planchettes : en travers, des multiples de 15 millimètres et, dans l'autre dimension, des multiples de 5 millimètres. Le tracé du tranchage est fait avec 3 millimètres de moins que les dimensions extérieures.

La profondeur de chaque cuvette est mesurée sur l'échantillon qu'elle doit contenir.

Après avoir été tracée au crayon, entaillée à mi-épaisseur avec un tranchet et découpée avec des ciseaux, la carte est transformée en cuvette par le relèvement de ses quatre côtés que l'on recouvre et que l'on borde solidement avec le papier bleu de la sorte employée pour le recouvrage de la carte et des planchettes.

L'étiquette consiste en un petit bloc de bois, couvert de papier blanc et collé en bas de la cuvette. Les dimensions de la face supérieure de ces blocs sont les mêmes que celles des étiquettes employées pour les planchettes.

Le dessous de la cuvette est recouvert, comme le dessous des planchettes, de papier blanc destiné aux annotations.

Tringles de séparation

Les planchettes et les cuvettes se rangent, dans les tiroirs, en colonnes séparées par des réglettes en bois, de 8 sur 8 millimètres d'équarissage. Ces réglettes maintiennent bien en place les colonnes de planchettes, mais elles prennent une place assez notable. Lorsqu'un tiroir est presque rempli, et qu'il faut, cependant, y intercaler de nouveaux échantillons, on peut remplacer ces réglettes volumineuses par des réglettes plus petites ou, même, par des bandes de carton, de 8 millimètres de largeur et de 1 millimètre d'épaisseur, recouvertes de papier lissé glacé blanc.

Dans chaque colonne, les planchettes se rangent les unes à la suite des autres, si elles sont toutes de même largeur. Dans le cas où les planchettes à réunir les unes auprès des autres sont de largeurs différentes, on peut chercher à réaliser des groupements tels que celui qui est représenté par la figure 6 (p. 24).

Planchettes de calage

Pour permettre les intercalations, on réserve, dans chaque tiroir, un certain nombre de colonnes que l'on ne garnit pas complètement d'échantillons. Afin d'empêcher tout déplacement des tringles de séparation qui limitent ces colonnes incomplètement garnies, on maintient leur écartement normal par des planchettes, non pourvues d'échantillons, qui servent, ainsi, simplement, de plan-

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chettes de calage. Une petite lame de bristol, collée sur le côté antérieur de celles des planchettes de calage qui maintiennent l'écartement des tringles de séparation, du côté antérieur des tiroirs, empêche ces planchettes de glisser vers l'arrière.

Pare-poussière

Le mode de division des meubles en casiers élémentaires, pourvus, sur cinq faces, de panneaux qui font obstacle à la circulation de l'air, le recouvrement des façades des tiroirs sur la devanture des casiers, et, surtout, la présence d'une feuille épaisse de feutre intercalée entre ces façades et les casiers, empêchent, efficacement, la pénétration de la poussière. On améliore encore ces bonnes conditions en plaçant, dans chaque tiroir, un morceau d'étoffe légère qui recouvre tout son contenu, et sur lequel se dépose la presque totalité de la petite quantité de poussière très fine qui a pu pénétrer dans l'intérieur du casier. J'emploie, à cet usage, des rectangles de satinette pour doublure, soyeuse, mince, très souple, de couleur noire, bordés, sur chacun de leurs quatre côtés, d'un ourlet de 12 millimètres, et dont les dimensions sont un peu plus grandes que les dimensions intérieures des tiroirs.

Pour les tiroirs ayant intérieurement

673 sur 546^{mm}

les pare-poussière doivent avoir

690 sur 565^{mm}

et, pour obtenir cette dimension, il faut découper la pièce d'étoffe en rectangles ayant

725 sur 600^{mm}

Avec ce mode de protection, la quantité de poussière qui se dépose, en vingt années, sur les planchettes d'échantillons, est à peine visible.

Classification des échantillons

Etiquetage des échantillons

L'étiquette collée en pied de chaque planchette porte, inscrites à l'encre de Chine (fig. 6) :

1° la détermination zoologique de l'échantillon,

2° la désignation du niveau géologique auquel il appartient,

3° l'indication de la localité où il a été recueilli,

4° s'il y a lieu, une petite croix, tracée dans l'angle inférieur droit de l'étiquette, signifiant qu'il y a des annotations inscrites au dos de la planchette.

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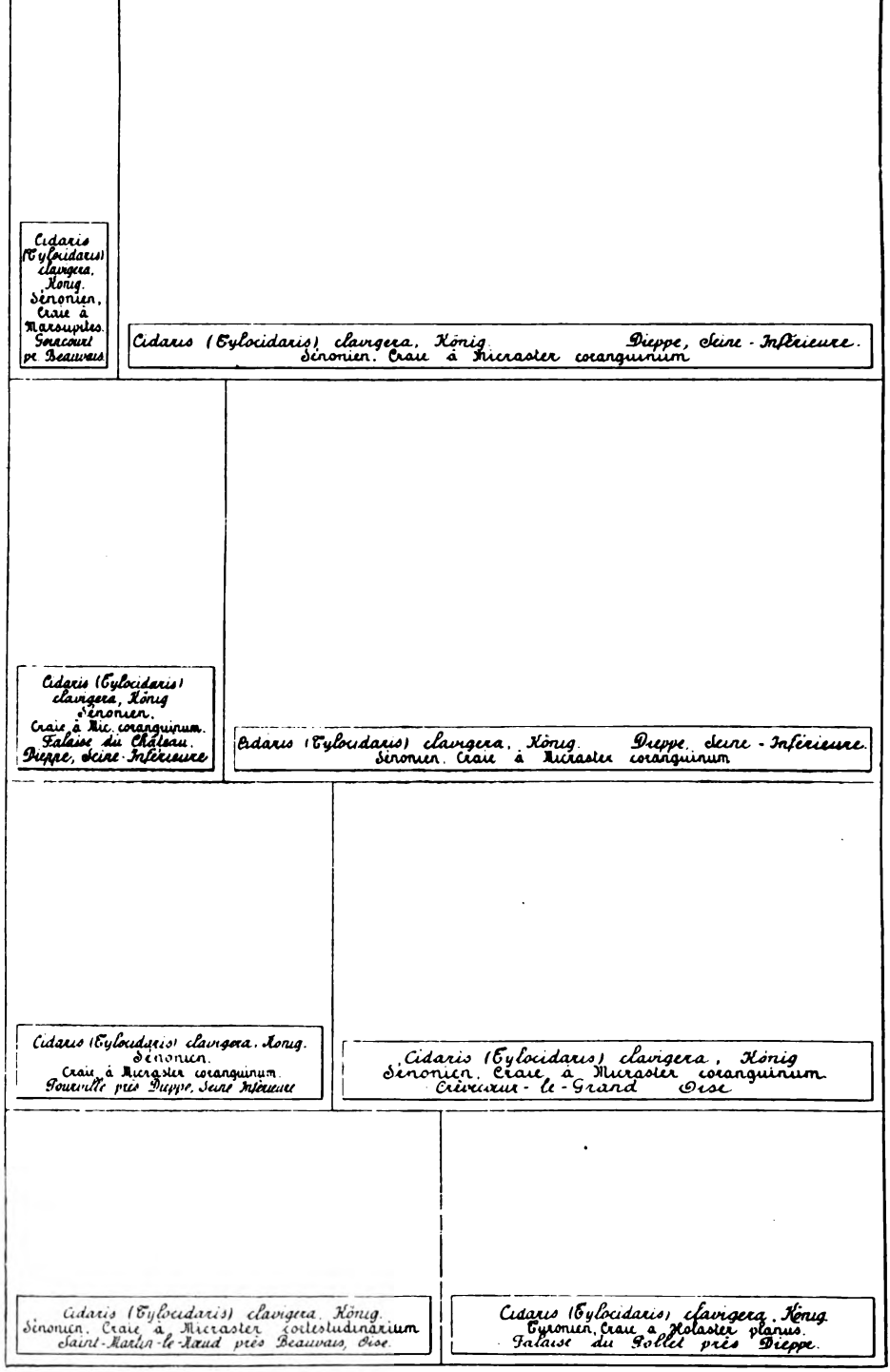


Fig. 6. — Exemple de l'étiquetage des planchettes. Grandeur d'exécution.

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Pour plusieurs motifs, et en particulier à cause de la constance des noms spécifiques et de la variabilité des noms génériques, il est, contrairement aux exemples indiqués sur la figure 6, préférable d'inscrire, d'abord, le nom spécifique et, ensuite, les noms génériques. C'est ce mode d'inscription que j'ai adopté, depuis quelques années, pour la collection en question. Afin d'éviter toute confusion, les noms génériques sont écrits avec des initiales majuscules, tandis que tous les noms spécifiques sont inscrits avec des initiales minuscules.

Planchettes de classification

Les échantillons sont classés dans l'ordre zoologique.

Les noms des groupes systématiques (Embranchements, Classes, Ordres, Tribus, Familles, Genres) sont indiqués, dans la Collection, par des inscriptions faites sur des petites planchettes de classification recouvertes de papiers de couleurs conventionnelles que l'on a eu soin de choisir parmi les couleurs les plus solides (fig.7). Chacune de ces planchettes de classification est placée en tête du groupe dont elle indique la dénomination.

Les noms des Sous-Embranchements, Sous-Classes, Sous-Ordres, etc., sont inscrits sur les mêmes couleurs que les noms des Embranchements, Classes, Ordres, etc., correspondants; mais ils sont, pour être distingués, précédés de deux points (: **Scutellinae**).

Lorsque l'un des groupes ou des sous-groupes précédents se divise en sections, les noms de ces dernières sont caractérisés par le signe /. C'est ainsi que l'inscription / : **Helicella** se rapporte à une section de sous-genre (genre *Helix*, sous-genre *Xerophila*, section de sous-genre *Helicella*).

Les planchettes de classification portent des numéros d'ordre qui précisent leur place dans la collection et chaque tiroir porte l'indication du numéro d'ordre de la première des planchettes de classification qu'il contient.

L'ensemble des échantillons appartenant à une même espèce est aussi précédé d'une planchette de classification indiquant le nom de l'espèce et le nom de son auteur.

Les planchettes de classification indicatrices des noms d'espèces ne portent pas de numéro d'ordre. Leur place dans la Collection est simplement déterminée par cette convention que, dans chaque genre, les espèces sont classées suivant leur ordre alphabétique. Dans le cas où un genre occupe plusieurs tiroirs, on indique, sur chacun de ces tiroirs, la première lettre du nom spécifique de la première espèce qu'il contient.

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Fig. 7. — Echantillons des papiers couleurs lissées, de teintes solides, adoptés pour les planchettes de classification.

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Le classement des espèces de chaque genre, suivant l'ordre alphabétique, est un système très commode, mais bien illogique, puisque, souvent, il éloigne des espèces qui sont voisines au point de n'être que des variétés, ou rapproche des espèces qui sont différentes au point d'appartenir à des sous-genres distincts.

Si l'on possédait une liste méthodique des espèces classées d'après leurs affinités réciproques, il y aurait certainement avantage à employer pour les espèces, comme pour les genres et les autres groupes, le système du numéro d'ordre qui est bien plus rationnel que l'ordre alphabétique.

Les numéros d'ordre de la liste des planchettes de classification doivent, soit parce que cette liste s'accroît, soit parce qu'elle subit des modifications systématiques, être changés de temps à autre. Mais comme ce changement est un travail long, qu'il faut éviter de recommencer trop souvent, on peut se contenter, pendant longtemps, de remaniements partiels du numérotage.

Pour simplifier ces remaniements partiels, on peut employer la méthode décimale qui permet d'intercaler, indéfiniment, entre deux nombres entiers consécutifs.

Si l'on a à intercaler deux genres entre le genre 115 et le genre 116 on pourra leur donner les numéros 115,3 et 115,6.

Si plus tard on a encore trois genres à intercaler entre les genres 115,3 et 115,6 on les numérotera, par exemple, 115,4, 115,5 et 115,55.

L'intercalation, par cette méthode décimale, est toujours possible. Les nombres décimaux

115
115,3
115,4
115,5
115,55
115,6
116

se trouvent, ainsi rangés, dans leur ordre de grandeur, et la place de l'objet désigné par chacun d'eux se trouve parfaitement déterminée, ce qui est, ici, l'unique but du numérotage.

Etiquetage des tiroirs

La façade de chaque tiroir (pl.6) est pourvue de trois porte-étiquettes, pour recevoir des étiquettes mobiles, en carte-bristol, ayant 110×70 millimètres lorsqu'elles doivent garnir entièrement le porte-étiquette.

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Le premier porte-étiquette (celui de gauche) est destiné à recevoir deux étiquettes superposées qui sont indépendantes l'une de l'autre afin de se prêter aux remaniements de l'étiquetage.

L'une de ces étiquettes, qui a 110×70 millimètres, porte le numéro indicateur de la place du tiroir (fig.8).

L'autre, qui a seulement 110×26 millimètres, et qui ne recouvre ainsi que la partie inférieure de l'étiquette précédente, porte le numéro d'ordre de la première planchette de classification contenue dans le tiroir et, s'il y a lieu, un nom d'embranchement.

Le deuxième porte-étiquette est destiné à recevoir les étiquettes portant les noms des Classes, des Ordres, des Tribus et des sous-groupes correspondants.

Le troisième porte-étiquette est destiné à recevoir les étiquettes portant les noms des Familles, des Genres, des Espèces et des divisions de ces groupes.

Les inscriptions sur les étiquettes des tiroirs sont faites sur des bandes de papier des mêmes couleurs conventionnelles que celles employées pour les planchettes de classification placées dans les tiroirs.

La figure 8 (p. 29) donne un exemple de l'étiquetage de deux piles consécutives de 20 tiroirs.

Dans cet exemple, les cinq premiers tiroirs de chacune des deux piles sont laissés vides, en prévision des remaniements éventuels des tiroirs voisins.

Tous les tiroirs portent le numéro de la case qu'ils occupent.

L'absence d'un numéro d'ordre de classification indique que le tiroir reste provisoirement vide et disponible pour les remaniements partiels qui seraient nécessités par l'intercalation de nouveaux échantillons.

Chaque nom d'embranchement, de classe, d'ordre, de tribu, de famille, de genre et d'espèce n'est indiqué qu'une fois, c'est-à-dire seulement sur le tiroir dans lequel commence le groupe considéré.

D'après ces diverses conventions, les inscriptions portées sur les trois étiquettes du sixième tiroir de la première des deux piles considérées signifient, en tenant compte des étiquettes en blanc des tiroirs suivants :

1° Que la place actuelle du tiroir est la 126e case.

2° Que la première des planchettes de classification contenues dans le tiroir a pour numéro d'ordre le nombre 2180.

3° Que tous les tiroirs des deux piles considérées sont affectés à l'embranchement des Mollusca.

4° Que tous les tiroirs des deux piles considérées sont affectés à la classe des Pelecypoda.

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123			143		
124			144		
125			145		
126 2180 MOLLUSCA	PELECYPODA Asiphonida MONOMYARIA	Ostreidae *Ostrea* a	146 2191		Inoceramidae *Gervillia* *Inoceramus* a
127 2180 (Suite)		d	147 2402		i *Perna*
128 2180 (Suite)		m	148 2417		Mytilidae *Mytilus* *Modiola*
129 2180 (Suite)		t	149 2423		*Lithophagus* *Lithodomus*
130 2190		*Alectryonia*	150 2449		*Crenella* *Dreissena*
131 2192		*Gryphaea* *Exogyra*	151 2452		Prasinidae
132 2213		Anomiidae	152 2469		Pinnidae
133 2235		Spondylidae	153 2518	HOMOMYARIA	Arcidae
134 2251		Limidae	154 2529		Pectunculidae
135 2274		Pectinidae *Pecten* a	155 2540		Nuculidae
136 2274 (Suite)		g	156 2587		Trigoniidae
137 2274 (Suite)		n	157 2603		Unionidae *Unio*
138 2307		*Vola* *Amusiopecten*	158 2643		*Anodonta*
139 2319	HETEROMYARIA	Aviculidae *Avicula*	159 2651	Siphonida INTEGRIPALLIATA	Astartidae *Cardita*
140 2325		*Melagrina*	160 2685		*Astarte* *Opis*

Fig. 8. — Exemple des inscriptions portées sur les étiquettes de deux piles consécutives de tiroirs.

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5° Que les tiroirs 126 à 158 sont affectés à l'ordre des Asiphonida.
6° — 126 à 138 — à la tribu des Monomyaria.
7° — 126 à 131 — à la famille des Ostreidae.
8° — 126 à 129 — au genre Ostrea.
9° Que le tiroir 126 est affecté aux espèces du genre Ostrea dont le nom spécifique commence par les lettres *a* ou *b* ou *c*.

Les inscriptions portées sur les étiquettes du septième tiroir de la 2e pile considérée signifient, en tenant compte des étiquettes des tiroirs précédents :

1° Que la place actuelle du tiroir est la case 147.

2° Que la première des planchettes de classification contenues dans le tiroir a pour numéro d'ordre le nombre 2402.

3° Que le tiroir est affecté à la suite : de l'embranchement des Mollusca, de la classe des Pelecypoda, de l'ordre des Asiphonida, de la tribu des Heteromyaria, de la famille des Inoceramidae.

4° Qu'il contient les dernières espèces du genre Inoceramus et le genre Perna.

Avec ce système d'étiquetage, une personne soigneuse, même complètement étrangère à la zoologie, peut, très facilement et sans crainte d'erreur, intercaler dans la collection tout lot d'échantillons déterminé génériquement et spécifiquement. Elle commence, d'abord, en cherchant le numéro d'ordre de chaque genre, à ranger sur une table, dans l'ordre zoologique adopté, tout le lot à classer. Elle cherche, ensuite, successivement, toujours au moyen des numéros d'ordre des genres, les divers tiroirs où se trouvent les places des échantillons donnés. Si l'on veut, par exemple (fig.8), trouver la place de l'espèce Inoceramus labiatus, il suffit de chercher le numéro d'ordre du genre Inoceramus, et de tenir compte de ce que dans chaque genre les espèces sont rangées par ordre alphabétique, pour trouver immédiatement que la place de l'espèce en question est dans le tiroir qui occupe la case 147.

Quant aux remaniements partiels nécessités par l'intercalation de nouveaux échantillons, ils sont rendus faciles par la présence des cinq tiroirs vides qui se trouvent en tête de chacune des deux colonnes.

Si, par exemple, le genre Perna vient à être représenté dans la Collection par un nombre d'échantillons suffisant pour occuper, à lui seul, un tiroir tout entier, il suffit, pour fournir à ce genre la place qui lui est nécessaire, de remonter d'une case le tiroir 146, qui devient ainsi le nouveau 145, et de mettre à la place 146, devenue libre, un tiroir pour recevoir les Inoceramus des espèces dont le nom commence par la lettre *l* ou par une des lettres suivantes.

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Pour mettre l'étiquetage des tiroirs d'accord avec ce changement il faudra :

1° Mettre sur les deux tiroirs occupant maintenant les cases 145 et 146 les étiquettes portant ces deux numéros indicateurs de leur nouvel emplacement ;

2° supprimer la lettre *l* qui se trouve sur l'étiquette de droite du tiroir 147 ;

3° inscrire cette même lettre sur l'étiquette correspondante du tiroir qui occupe maintenant la case 146.

Si, plus tard, il faut avoir deux tiroirs au lieu d'un pour loger la famille des Pectunculidae, précédemment contenue tout entière dans le tiroir qui occupe la case 154, il suffit de faire remonter d'une case ce tiroir et les neuf précédents et la case 154, ainsi libérée, recevra un tiroir vide qui servira à loger la seconde moitié des genres composant la famille en question.

Pour mettre l'étiquetage des tiroirs d'accord avec ce changement il faudra :

1° remanier, sur les dix tiroirs remontés, les étiquettes indicatrices du numéro de la case occupée par chacun d'eux ;

2° ajouter à la suite du mot Pectunculidae, sur l'étiquette de droite du tiroir qui occupe maintenant la case 153, les noms des genres contenus dans ce tiroir ;

3° mettre, dans le porte-étiquette de gauche du tiroir, qui occupe maintenant la case 154, d'abord l'étiquette portant ce numéro 154, indicateur de la place occupée par le tiroir et ensuite le numéro d'ordre du premier genre contenu dans le tiroir ;

4° inscrire sur l'étiquette de droite de ce même tiroir les noms des genres qu'il contient.

Contrairement à ces deux exemples, les remaniements destinés à fournir la place nécessaire aux intercalations doivent être faits, de préférence, par transport des échantillons vers les tiroirs qui suivent plutôt que par transport vers les tiroirs qui précèdent celui qui fait l'objet du remaniement.

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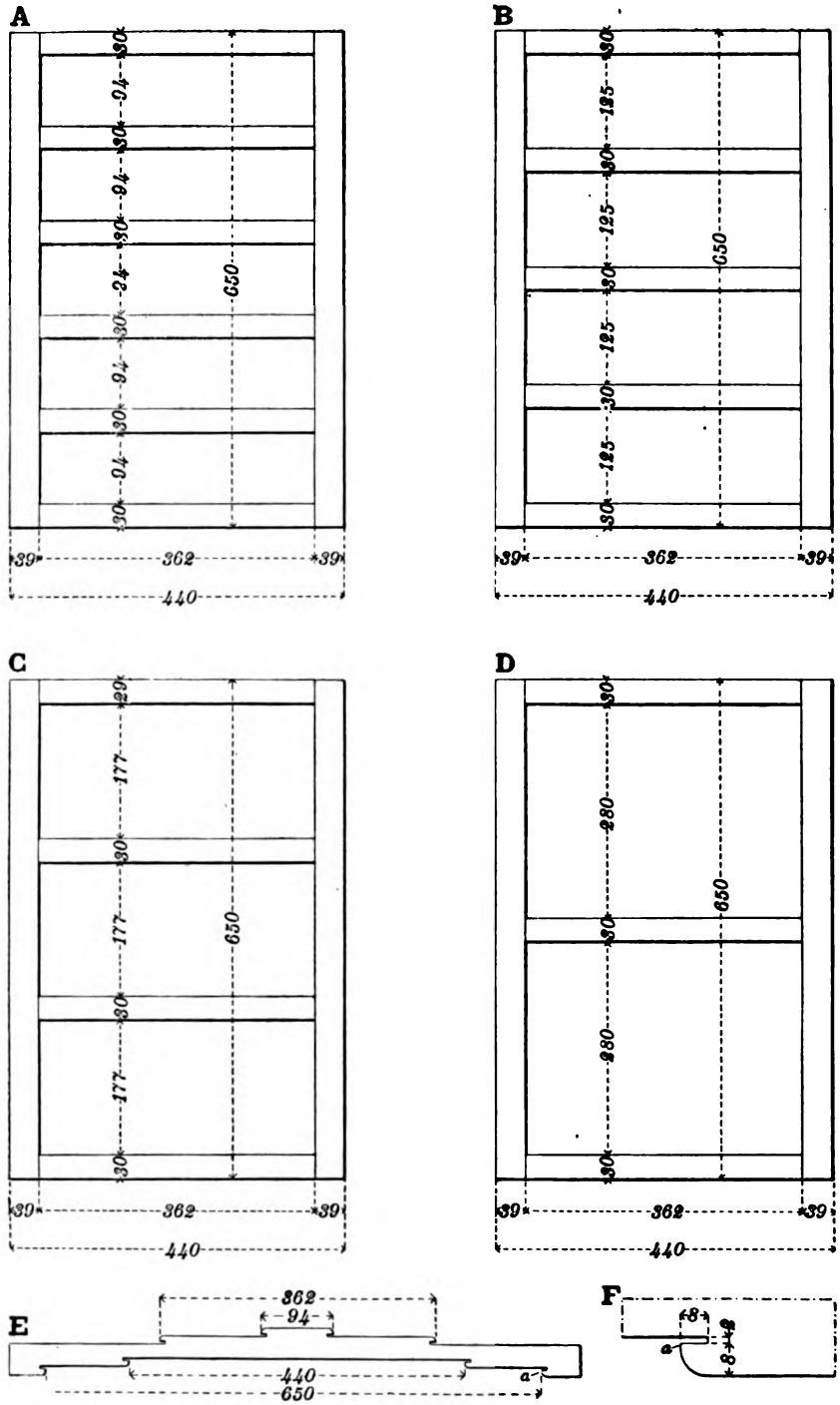


Fig. 9. — A, B, C, D, Façades des casiers pour les tiroirs servant au rangement du matériel de la Collection. Ech. 0,1.

E. Gabarit en tôle de 1mm 1/2 d'épaisseur, servant à vérifier les quatre dimensions principales des casiers représentés en A. Ech. 0,1.

F. Détail d'une butée. Ech. 0,5.

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MEUBLES POUR LE RANGEMENT DU MATÉRIEL

J'emploie, pour le rangement du matériel de la Collection paléontologique dont il vient d'être question (approvisionnement de planchettes pour coller les échantillons, de planchettes de classification, de tringles de séparation, etc.), et pour le rangement de tout le petit matériel de mon laboratoire, des meubles à tiroirs identiques à ceux qui viennent d'être décrits et, aussi, des meubles plus petits.

Les casiers qui forment les éléments de ces derniers ont la même hauteur et la même profondeur extérieures que les casiers pour petits échantillons de la Collection paléontologique, mais ils sont plus étroits. Ils ont :

Dimension	transversale	extérieure	440	millimètres
—	verticale	—	650	—
—	sagittale	—	605	—

Ils sont, comme le représente la figure 9, à 2, 3, 4 ou 5 cases.

Comme les tiroirs pour les petits échantillons de la Collection paléontologique, les tiroirs de ces casiers ont 546 millimètres de profondeur intérieure, mais ils n'ont que 338 millimètres de largeur intérieure.

La hauteur intérieure de ces tiroirs est de

76	millimètres	pour les casiers à	5	cases
107	—	—	4	—
159	—	—	3	—
262	—	—	2	—

Les dispositions adoptées pour leurs façades sont indiquées par les figures C à F (page 7).

Les dimensions de ces casiers et de ces tiroirs sont indiquées dans les quatre dernières colonnes du tableau de la page 34.

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TABLEAU DES DIMENSIONS DES TIROIRS ET DES CASIERS

	Désignation des Dimensions			Meubles pour le classement de la Collection paléontologique		Meubles pour le rangement du Matériel			
				Meubles pour les échantillons moyens	*Meubles pour les petits échantillons*	*Casiers à 5 tiroirs*	*Casiers à 4 tiroirs*	*Casiers à 3 tiroirs*	*Casiers à 2 tiroirs*
TIROIRS	Corps du tiroir.	Dimensions intérieures...	Largeur.	713	673	338	←	←	←
			Profondeur.	607	516	←	←	←	←
			Hauteur.	107	76	←	107	159	262
		Dimensions extérieures..	Largeur.	733	693	358	←	←	←
			Profondeur.	631	570	←	←	←	←
			Hauteur.	122	91	←	122	174	277
		Epaisseur des bois.....	Côtés.	10	←	←	←	←	←
			Devant.	14	←	←	←	←	←
			Derrière.	10	←	←	←	←	←
			Fond.	10	←	←	←	←	←
		Largeur des bois.....	Côtés.	122	91	←	122	174	277
			Devant.	107	76	←	107	159	262
			Derrière.	97	66	←	97	149	252
		Profondeur de la rainure en pente creusée dans les côtés pour recevoir les fonds..	en avant...	6	←	←	←	←	←
			en arrière..	2	←	←	←	←	←
	Devanture rapportée.	Longueur.		769	729	394	←	←	←
		Largeur.		145 (147)	114 (116)	←	145(147)	197(200)	300(305)
		Epaisseur.		12	←	←	←	←	←
		Saillies sur le corps du tiroir..	en haut.	13 (14)	←	←	←	13 (15)	13 (16)
			en bas.	10 (11)	←	←	←	←	10 (12)
			sur les côtés.	18	←	←	←	←	←
	Ecartements horizontaux depuis l'axe vertical de la devanture jusque		à l'axe des Vis en fer....	99et177	←	48et126	←	←	←
			au dehors des Porte-étiquettes	201	←	148	←	←	←
			au centre des Anneaux.....	275	←	0	←	←	←
	Ecartement vertical depuis l'axe horizontal de la devanture jusqu'à l'axe des Vis en fer.			21	←	←	←	←	←
	Distance des bords des devantures rapportées, jusqu'à l'axe des Vis en cuivre.			40	»	»	»	40	←
CASIERS	Dimensions extérieures des Casiers.		Largeur en avant.....	815	775	440	←	←	←
			— en arrière.....	813	773	439	←	←	←
			Hauteur..	650	←	←	←	651	650
			Profondeur.	666	605	←	←	←	←
	Dimensions intérieures des Cases.	Largeur. ...	à l'entrée des Cases.....	737	697	362	←	←	←
			entre les Joues des coulisseaux à l'arrière.....	747	707	372	←	←	←
		Hauteur. ...	à l'entrée des Cases.....	125	94	←	125	177	280
			entre les Coulisseaux....	133	102	←	133	185	288
		Profondeur.		646	585	←	←	←	←
	Equarrissage des Montants et des Traverses.	Montants.	de la façade.	39 × 60	←	←	←	←	←
			de l'arrière..	29 × 60	←	←	←	←	←
		Traverses supérieures et inférieures.	de la façade.	30 × 60	←	←	←	←	←
			de l'arrière..	25 × 60	←	←	←	←	←
			des côtés....	29 × 60	←	←	←	←	←
		Traverses séparatives des Cases.		30 × 30	←	←	←	←	←
	Epaisseur des 5 panneaux (2 côtés, 1 dessus, 1 dessous, 1 derrière).			10	←	←	←	←	←
	JEU des Tiroirs dans les Cases.	en largeur...	en avant à l'entrée des Cases.....	4	←	←	←	←	←
			en arrière entre les joues des Coulisseaux	14	←	←	←	←	←
		en hauteur..	en avant à l'entrée des Cases.....	3	←	←	←	←	←
			en arrière entre les Coulisseaux.....	11	←	←	←	←	←
		en profondeur.....		15	←	←	←	←	←

Le signe ← signifie : *même dimension que celle qui précède, sur la même ligne.*

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EXPLICATION DES PLANCHES

Echelle des figures 1 à 8..... 0,3
Echelle de la figure 9..... 1/15
Echelle des figures 10 et 11..... 0,02

Planche 1

Fig. 1. — Casier à tiroirs pour le rangement des échantillons d'une petite Collection paléontologique. — Vue de face. Deux tiroirs ont été représentés.

Planche 2

Fig. 2. — *Idem.* — Coupe horizontale suivant le plan A. Le tiroir est représenté non coupé.

Planche 3

Fig. 3. — *Idem.* — Coupe verticale suivant le plan B parallèle à la façade. On a figuré deux tiroirs. La moitié droite du dessin représente la partie postérieure du casier. La moitié gauche du dessin est une vue de la coupe regardée en sens inverse de la précédente et représente, par conséquent, la partie antérieure du casier.

Planche 4

Fig. 4. — *Idem.* — Coupe verticale suivant le plan antéro-postérieur ou sagittal C. Deux tiroirs ont été figurés. L'un est représenté en coupe et l'autre est représenté non coupé.

Planche 5

Fig. 5. — Soubassement et table de casiers, semblables à celui représenté par les planches précédentes, groupés pour former des tables-comptoirs de 80 centimètres de hauteur. — Coupe suivant le plan vertical antéro-postérieur D de la figure 6.

Fig. 6. — *Idem.* — Vue de face.

Fig. 7. — Soubassement et corniche de grands meubles formés par l'empilement de quatre assises de casiers semblables à celui représenté par les planches précédentes. — Coupe suivant le plan vertical antéro-postérieur E de la figure 8.

Fig. 8. — *Idem.* — Vue de face.

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Planche 6

Fig. 9. — Grand meuble formé par l'empilement de quatre assises de casiers semblables à celui représenté par les planches 1 à 4. — Vue de face.

Planche 7

Fig. 10. — Exemple de l'installation dans une salle de $9^{m}72$ sur 11 mètres de grands meubles et de tables-comptoirs des types représentés sur les planches 5 et 6. — Plan.

Fig. 11. — Coupe suivant M N.

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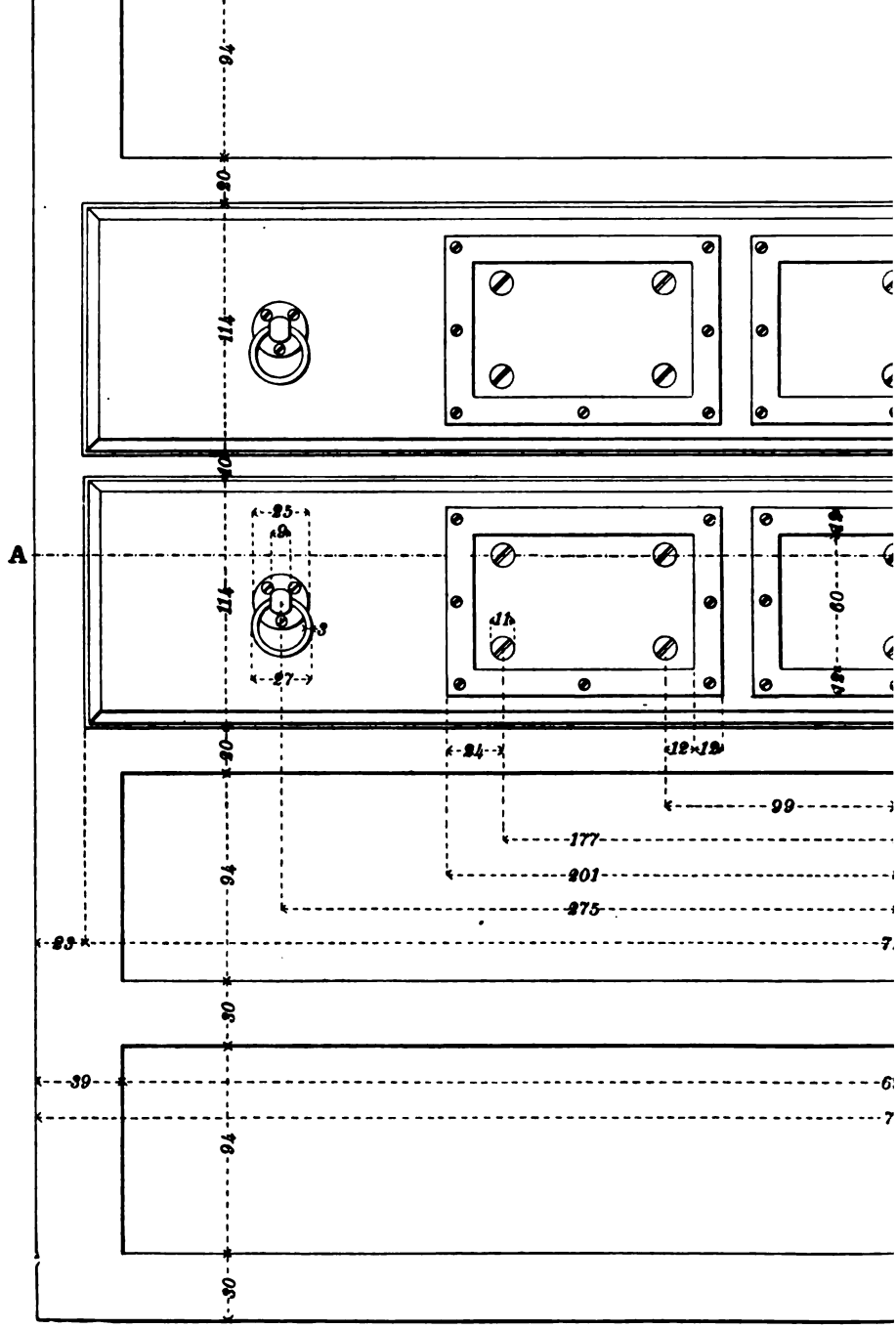
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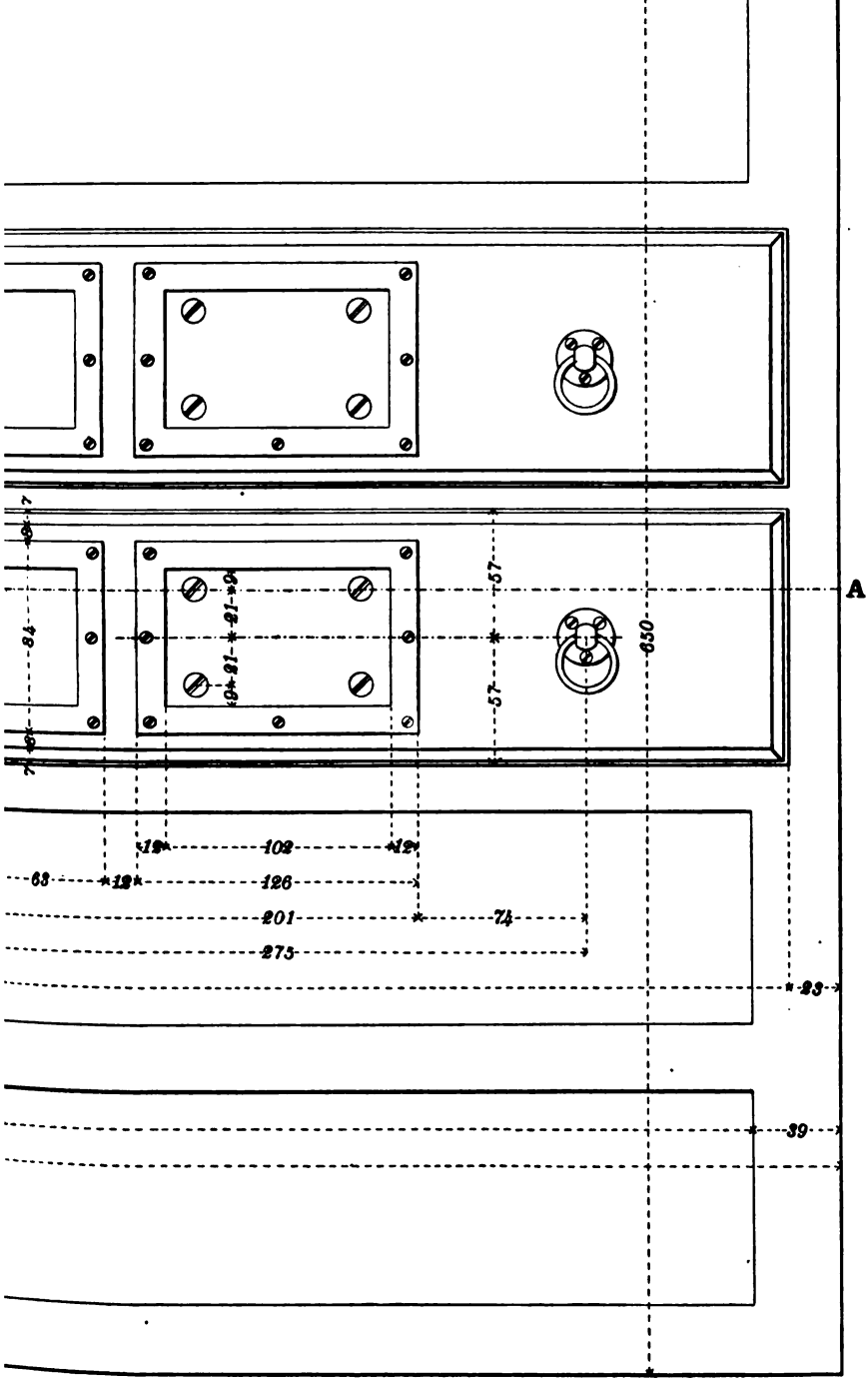
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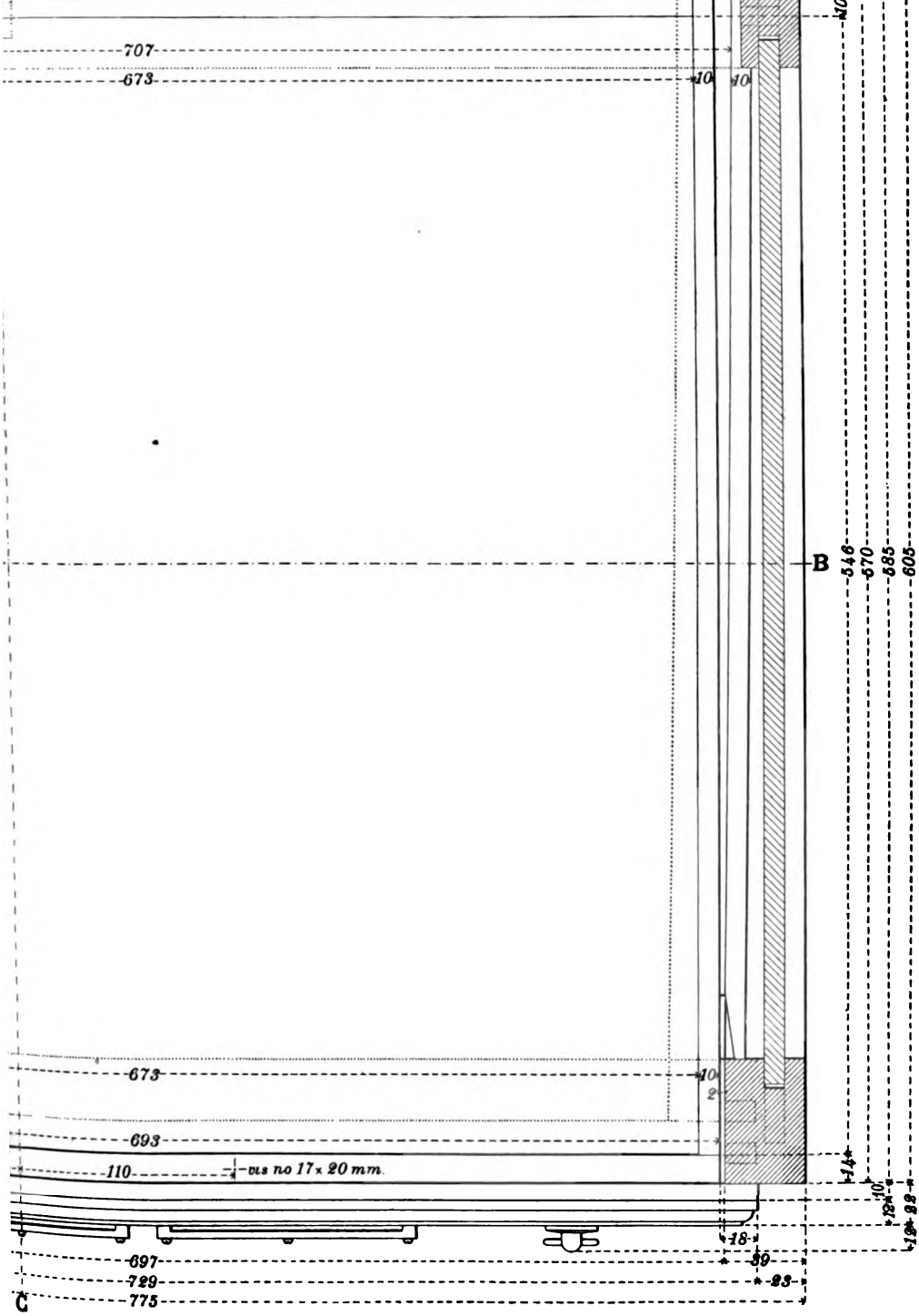


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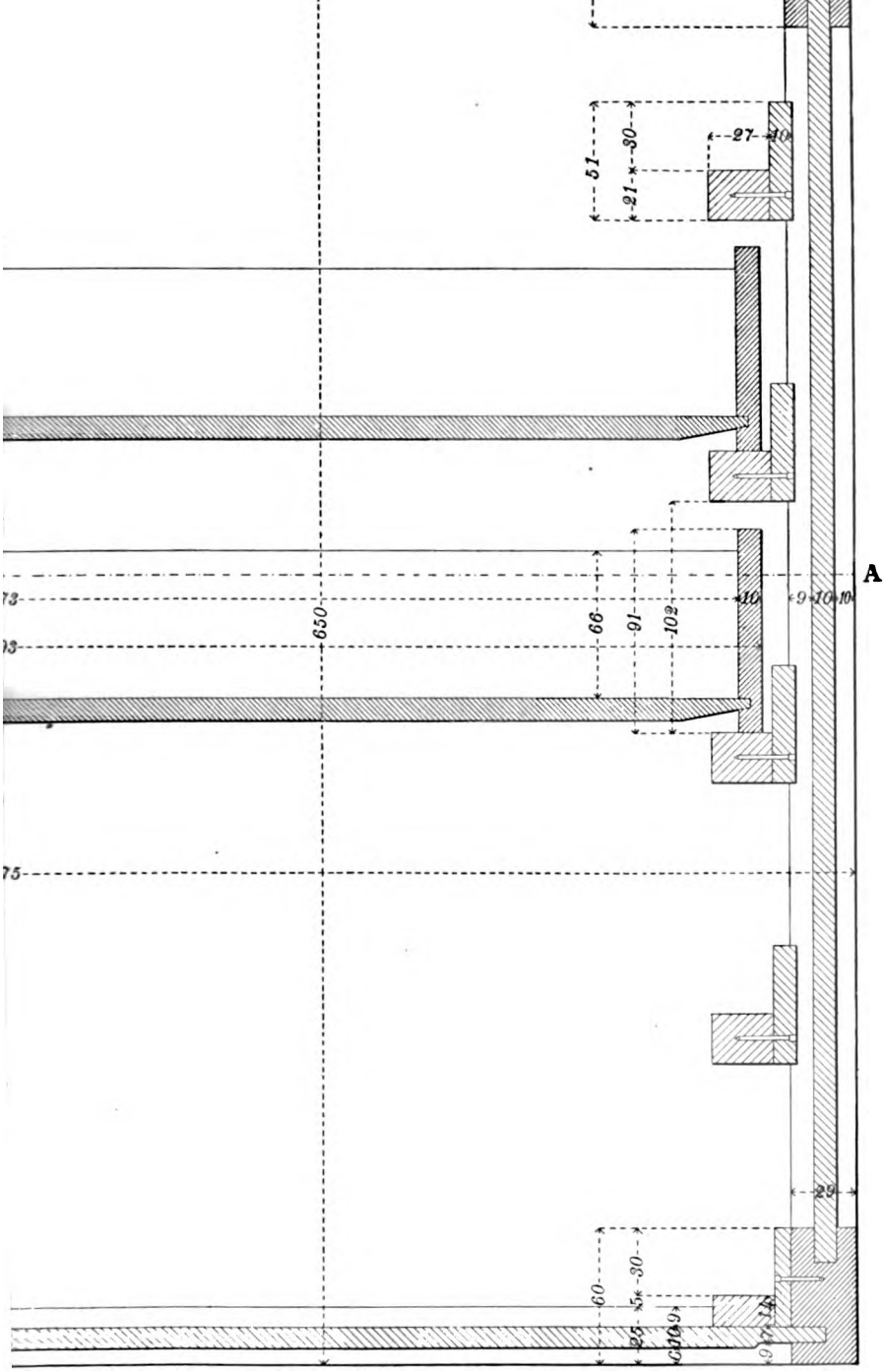
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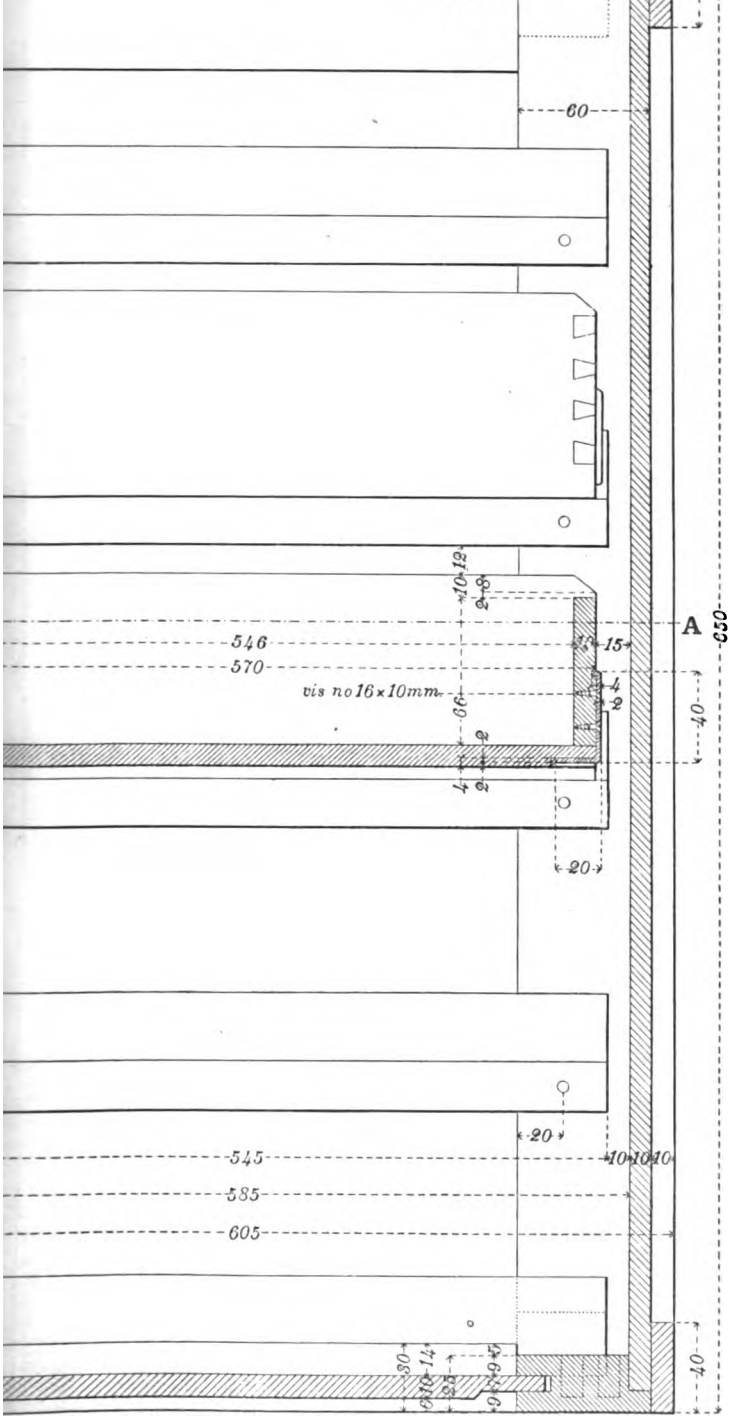
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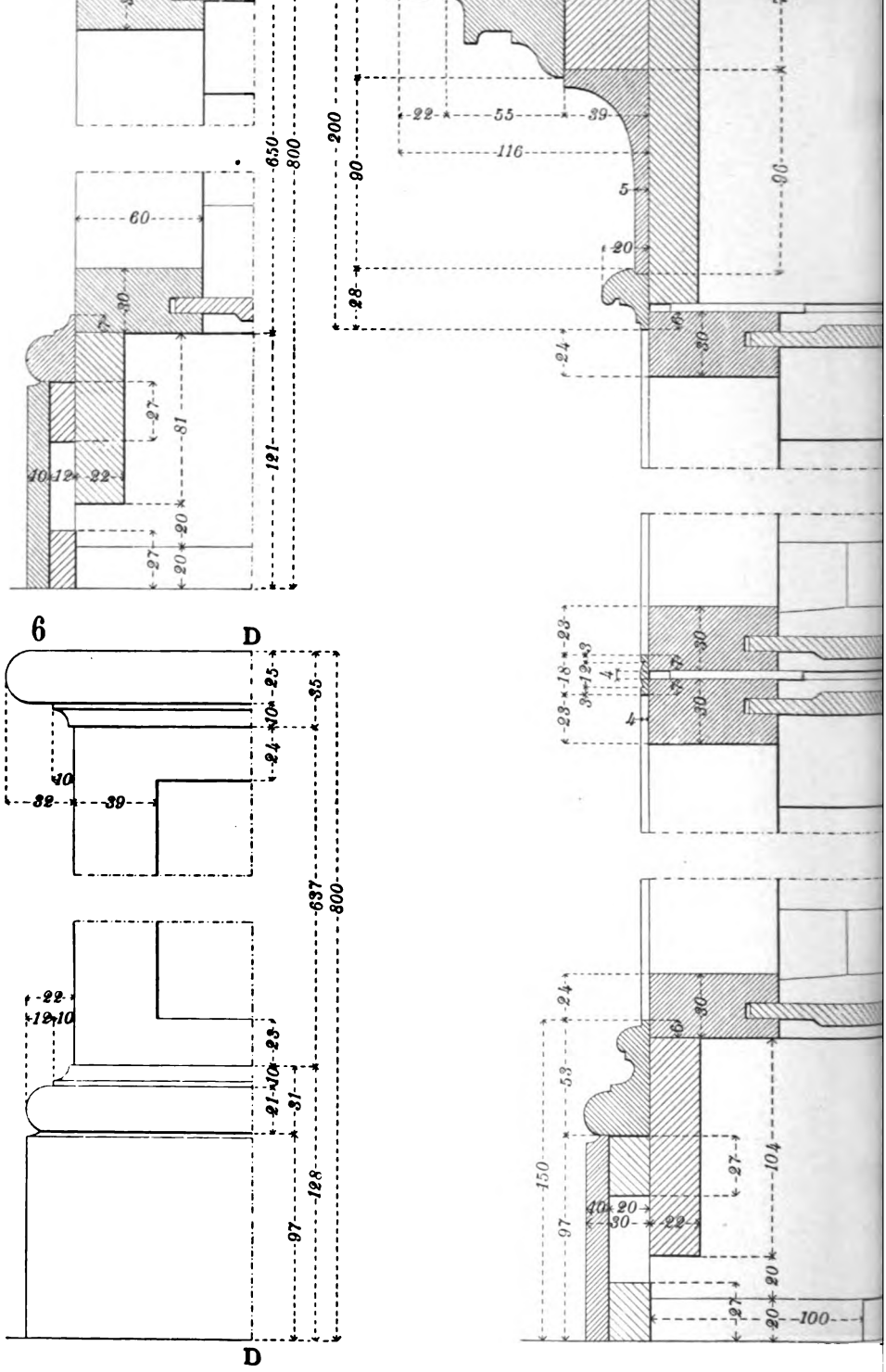


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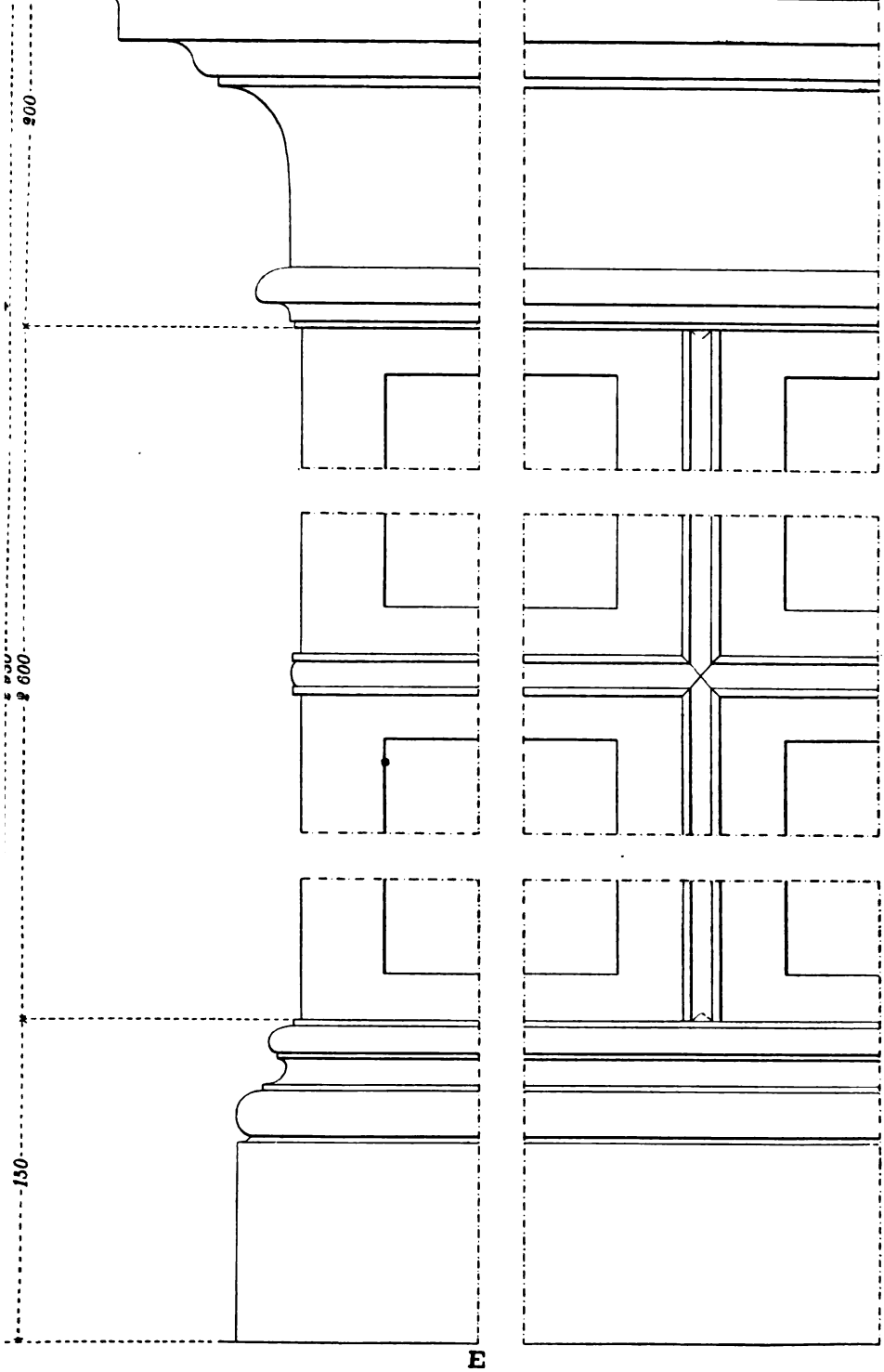
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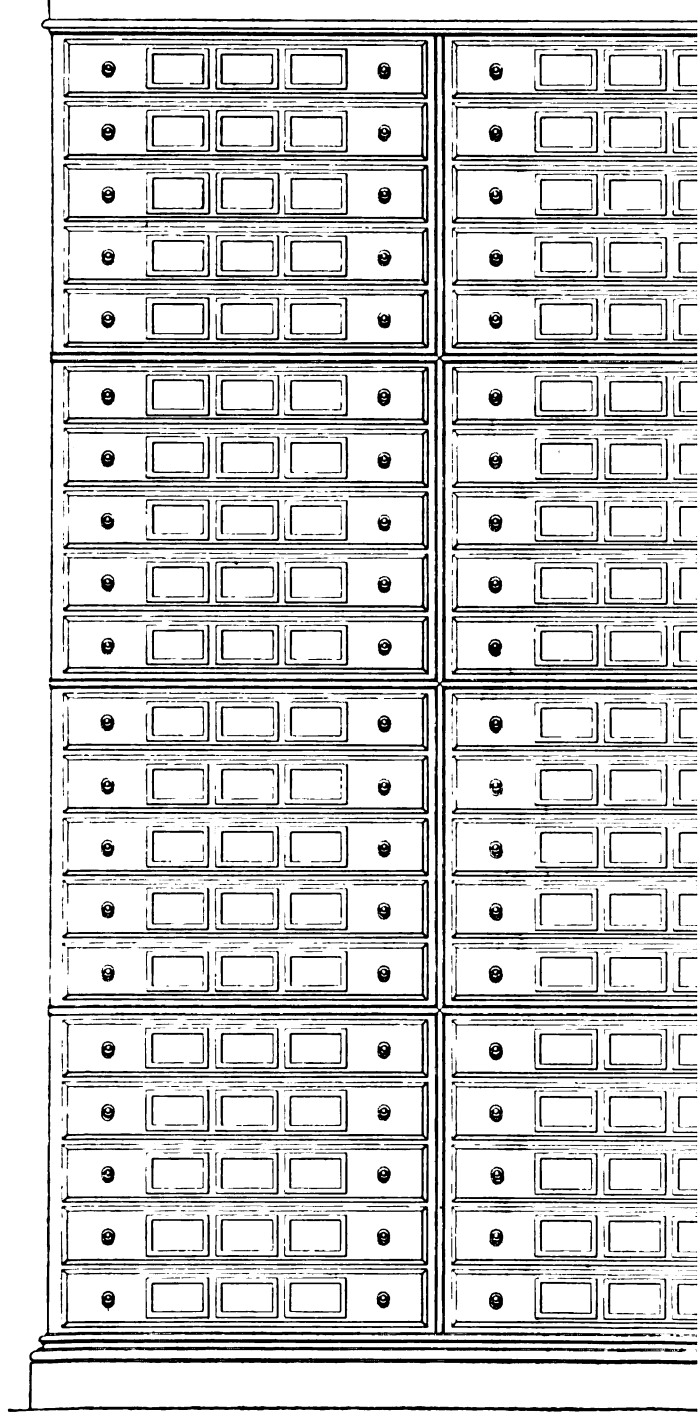
Casiers
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Casiers à tiroirs
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Exemple de groupement des Casiers



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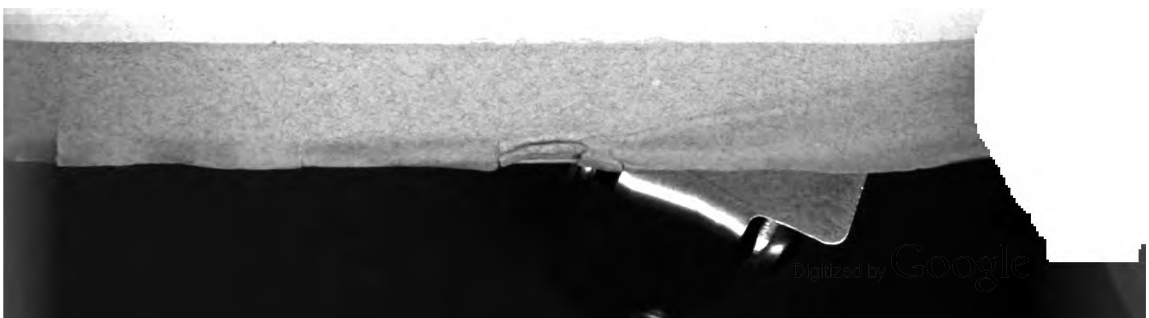
On the presence of mobile fat in the Chromatophores of the Crustacea (Hippolyte varians).

By Frederick Keeble, University College Reading, and F. W. Gamble, Owens College Manchester.

The present paper is an abstract of our researches into the distribution and movements of fat in the prawn *Hippolyte*.

The researches have been carried on at Trégastel (Brittany), at Naples, and in the laboratories of Owens College Manchester, and of University College, Reading.

1) Distribution of Fat. *Hippolyte* offers a remarkable contrast to crabs, lobsters, and cray-fish with respect to the distribution of its fat. In these latter, according to Dastre (1901), storage-fat is confined to the liver: in *Hippolyte* we find that it occurs not only in liver, but also in the chromatophores and epidermal cells. As means of identification, we use 1) the optical characters of the granules, 2) the osmic acid reaction, 3) that of Sudan 3. and Scharlachrot (Michaelis methods).


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jelly the chromatophores show, in addition to red, orange and blue pigments, the granular fat associated with, but distinct from, these pigments.

The fat in the form of spherical colourless granules .5—1 μ in diameter, lies either in the centres or in the branches of the chromatophores. In a green *Hippolyte*, for example, the fat granules run in rows which follow the lines of the branching and anastomosing chromatophores in which they occupy separate tracts.

2) Movements of the Fat of the Chromatophores. Like the pigments themselves, the fat of the chromatophores is mobile. At night when the animals are in the nocturnal phase (Gamble and Keeble, 1900) all pigments being contracted to the centres, the fat is likewise aggregated in the centres. When the pigments pass into their branches, at daybreak, the fat also passes into its branches. When the pigments are fully expanded and occupy the finest intercellular ramifications, the fat appears in the form of a fine net-work — a lacteal system — about the terminations of the chromatophores in skin, muscle and connected tissue. Though, broadly speaking, fat-movement and pigment-movement correspond, the correspondence is not absolute, nor does artificial stimulation always affect pigment and fat alike.

3) The occurrence of fat in relation to food and to illumination. Fed and starved *Hippolytes* show the same general phenomena of fat distribution. The fat of the chromatophores appears, however to be independent of the food supply.

Light, on the other hand, exerts a powerful influence on the occurrence of fat in the chromatophores. Thus, *Hippolytes* taken from the sea and exposed for an hour to bright sunlight, shew a denser network of finer mesh than do controls kept in diffuse light.

Continued darkness produces, after five to eight days, depletion of the chromatophoric fat in male and immature female specimens. Depletion in mature females occurs in from eight to ten days; in large *Hippolyte viridis*, the process may even then be incomplete.

Such dark-kept specimens and controls kept for the same length of time in diffused light, exposed for three hours to direct sunlight showed dense networks of large colourless fat-granules in both cases.

4) Summary. The Chromatophores of *Hippolyte* normally contain colourless fat distributed in tracts separate from those occupied by the red and yellow pigments. The fat exhibits movements very similar to those of the diurnal pigments. These movements bring the fat from the chromotophore-centres to the inter-cellular spaces of the


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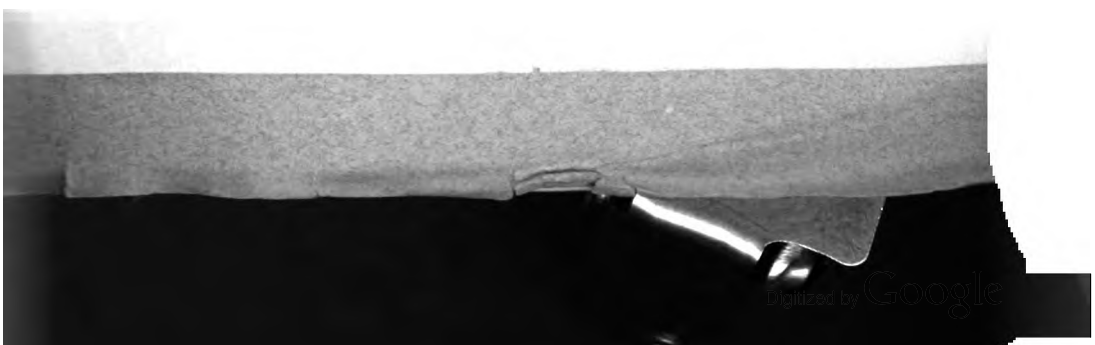
conditions the amount of fat diminishes but little in diffuse light, whereas, in darkness, the fat disappears, and this whether food is supplied or not. Both light- and dark-kept specimens exhibit dense networks of fat after a short exposure to bright light.

A fuller statement and a consideration of the conclusions drawn from these experiments is in preparation.

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ON THE COAGULATION OF THE BLOOD OF SOME ARTHROPODS AND ON THE INFLUENCE OF PRESSURE AND TRACTION ON THE PROTOPLASM OF THE BLOOD CELLS OF ARTHROPODS.

LEO LOEB.

[Reprinted from BIOLOGICAL BULLETIN, Vol. IV., No. 6, May, 1903.]

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ON THE COAGULATION OF THE BLOOD OF SOME ARTHROPODS AND ON THE INFLUENCE OF PRESSURE AND TRACTION ON THE PROTOPLASM OF THE BLOOD CELLS OF ARTHROPODS.[1]

LEO LOEB.

(From the Biological Laboratory, Woods Holl, Mass., and from the J. H. L. Molson Laboratories of Pathology and Bacteriology, McGill University, Montreal.)

I The Normal Coagulation of the Blood in Some Arthropods.

For this work the blood of *Limulus polyphemus*, *Homarus Americanus*, *Platyonychus ocellatus*, and some other arthropods, was used.

Coagulation of the blood takes place in a somewhat different way in *Limulus*, lobster and spider crab.

In *Limulus* the blood coagulates as a bulky mass which in the following 24 hours contracts considerably. A second coagulation of the fluid expressed from the clot may take place, to a slight extent, forming very loose gelatinous masses around the central clot, or more rarely, the whole serum may afterward coagulate.

In the lobster the first coagulum is usually relatively small, consisting of shreds; usually however, in the course of twenty minutes to an hour, the serum remaining after the first coagulation coagulates as a solid gelatinous mass which in the next twenty-four hours retracts but little. Very rarely no coagulation of the serum remains after the first coagulation takes place.

In the spider or lady-crab, the first coagulation is similar to that of the lobster blood. No second coagulation however takes place.

A combination of the following factors comes into play in producing the clotting of blood in arthropods.

[1] This communication is in most parts only an abstract of a fuller report which shall appear later. It is intended to continue these experiments.

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1. *The Agglutination of Blood Corpuscles.*—This phenomenon may be observed uncomplicated by the other factors, *e. g.*, if we collect blood in distilled water, in solutions of gelatine or of adrenalin. Under these conditions the cytoplasm of the cells itself furnishes a sticky substance necessary for agglutination. Coagulation of the blood of arthropods is partially based on *the agglutination of the blood cells taking place spontaneously outside of the body.*

2. Just as the cells agglutinate, so the cell protoplasm having left the ruptured cell body agglutinates, and the remaining part of the cell frequently has the appearance of still intact but smaller cells. The protoplasm of cells which have been absolutely dissolved may also agglutinate and form gelatinous masses.

3. About six to ten minutes after the shedding of the blood the blood cells, which in the meantime have been mostly collected in strings, begin to move in contact with solid bodies, *e. g.*, the surface of the slide; the protoplasm of the cell becomes more and more drawn out, so that ultimately a fine network of flattened cells is formed in which for a certain time the anastomosing exoplasm can be distinguished from the granular endoplasm.

4. There also takes place the coagulation of a substance which previously was in solution. This coagulation is especially marked in the blood of the lobster, but it also takes place to a less degree in the blood of *Limulus.*

II. On Some of the Factors Determining the Second Coagulation of the Blood of the Lobster.

The conditions under which the coagulation of the substance first in solution in the blood of arthropods takes place can best be studied in the blood of the lobster. If, after the shedding of the blood, one quickly collects the first fibrin by shaking the vessel and then removing it with needles, the coagulation can be inhibited for longer or shorter periods, or even be entirely prevented. The fact that coagulation nevertheless usually takes place, although somewhat later, is partially to be explained by the fact that it is impossible to remove all shreds. These shreds mostly consist of agglutinated cells. If one examines the second gelatinous coagula formed at the bottom of the dish, even after

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having apparently most carefully removed the shreds, one frequently sees under the microscope a small mass of blood corpuscles which had not been removed from the blood, in the center of a small gelatinous coagulum.

In many cases, however, a second coagulation after having removed most of the shreds does not take place even if some of these shreds have remained. In the cases in which it does take place it is also possible that the cell protoplasm, derived from the solution of so many blood cells, may be sufficient to cause coagulation even if the absolutely intact blood cells themselves have been entirely removed. It may be found that by adding water to the blood during the first coagulation and afterwards filtering the diluted blood, the second coagulation can be inhibited with quite a certainty for a longer period than otherwise, or may be even entirely prevented. Dilutions of the blood therefore produce an absolute diminution in the formation of fibrin for a certain period, not only a relative one in proportion to the remaining fluid.

In the experiments made such diluted serum was afterwards distributed in small dishes, 4 or 5 cc. into each dish; different substances were added to several of those dishes to determine the influence of these substances on coagulation.

It could be shown that the addition of about two or three pieces of shreds of fibrin derived from the first coagulation of the lobster blood had a very pronounced effect on the coagulation of the diluted serum. Without the addition of such fibrin coagulation either did not take place at all, or only after some hours or on the next day. The difference in the dishes with shreds of fibrin and those without the fibrin was very marked. The influence of these shreds derived from the first coagulation was a localized one, the clotting always beginning directly around the pieces of fibrin and from here advancing concentrically towards the periphery of the fluid.

The fibrin however produced by the first coagulation is not the only substance having such an effect; pieces of muscle of the lobster act about equally well. In these experiments the muscle was carefully washed so that all blood was removed. The fibrin of the blood of rabbits or rats, or muscle of frogs, was without

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effect. The fibrin of these latter animals was used as the fibrin of the lobster, or in some instances was for two hours put into lobster serum previous to the experiment, so that substances present in the fibrin which might inhibit coagulation were removed. Lobster fibrin, treated in this way, was active; the fibrin of rats and rabbits was without effect. If, however, to the lobster serum previously treated with fibrin of rabbits, fibrin of lobster was added, the serum coagulated; therefore, no substance inhibiting coagulation had been extracted from the mammalian fibrin.

Small pieces of the pancreas-liver of the lobster entirely prevented, as was to be expected, coagulation.

After pieces of the first lobster fibrin had been put for ten minutes or longer into absolute alcohol, then washed and dried, they lost almost entirely their power to produce coagulation. A trace of the second gelatinous coagulum, however, was sometimes still formed around such pieces. Control experiments showed that alcohol passing into the serum was not the cause of this loss of efficiency.

Pieces of fibrin put into chloroform water over night and even for three days, may still be very efficient, though not quite as active as fresh fibrin. Put into chloroform water for five days they lost to a large extent their efficiency. The addition of 1 cc. of chloroform water to 4 or 5 cc. of serum, or of a few drops of pure chloroform, to the serum had no more influence on the coagulation than the addition of an equal amount of water.

Heating of the fibrin in the water-bath to 46 or 47° for 30 to 40 minutes, destroyed its efficiency to a great extent, though not completely. Heating up to 51–54° for 40 minutes, destroyed its efficiency entirely or almost so.

Heating of the serum to 46–50° during 30 minutes, usually prevented the spontaneous coagulation of the serum. If, however, one adds to such serum previously heated, fresh fibrin, the serum coagulates almost as rapidly as normal serum.

The addition of 1 cc. of glycerine to 4 cc. of serum did not have any specific inhibiting influence. Also the addition of 1 cc. of a weak solution of pure gelatine did not have any specific influence.

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The addition of 1 cc. of water and 6–4 per cent. sodium chloride solution to the serum had in most cases but a slightly inhibiting effect.

In such solutions coagulation has occurred occasionally even more rapidly than with the pure serum; an accidental admixture of a larger number of blood cells to this liquid may perhaps have been the cause. All these experiments, as well as most of the following ones, were made with the addition of shreds of fibrin obtained from the first coagulation of the lobster blood.

The addition of 1 cc. of a 1 per cent. solution of potassium cyanide or of a 16 per cent. solution of Witte's peptone, or of a saturated solution of urea, had a decidedly inhibiting effect. The peptone solution prevented the coagulation entirely; the potassium cyanide and urea solutions frequently had only an inhibiting action for some time, after perhaps 16 hours (sometimes earlier) coagulation usually occurring. Generally only half an hour after the addition of these substances their effect became noticeable. The order in which these substances, according to their strength, acted on the serum, was the following: Potassium cyanide had the weakest and Witte's peptone the strongest action; urea stood between these substances.

If, however, the experiments were made in this way, that the pieces of the first fibrin were in the beginning put for some time into potassium cyanide, urea or peptone solutions, then after having been washed out, put into dishes containing the blood serum, the order of the loss of efficiency of the fibrin was reversed. Pieces having been kept for one half hour to two hours in peptone solution, were almost as effective as fresh fibrin, even pieces having been kept in peptone over night were frequently still very active, although in many cases they had lost a considerable part of their efficiency. It is of importance that the peptone be well washed out for some hours, otherwise the peptone, becoming extracted and dissolved in the serum, prevents its coagulation.

Pieces having been kept for one half to two hours in a solution of urea and having been afterwards well washed, have lost the greatest part of their efficiency, and after having been kept for twelve hours have become absolutely ineffective.


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A 1 per cent. solution of potassium cyanide usually destroys the efficiency of the fibrin inside of half an hour. After having been kept for two hours in such a solution the fibrin becomes opaque.

One cubic centimeter of a 2 per cent. solution of potassium oxalate, or 5 drops of a 10 per cent. solution of the same solution added to 4 cc. of serum, entirely prevents the coagulation.

One half cubic centimeter of a 2 per cent. solution of potassium oxalate inhibits the coagulation markedly.

The addition of 5 drops, or 1 cc., or 2 cc. of a 2 per cent. calcium chloride solution had, in one series of experiments, a slightly accelerating influence on the coagulation in comparison with control experiments which were made with the addition of 1 cc. of a weak sodium chloride solution. Calcium is probably present in a sufficient quantity in the serum and the farther addition of it can no longer exert a pronounced influence.

A more extended series of experiments on the action of different salts on the coagulation is to be made later. Some experiments have, however, already been made on the action of ammonium chloride (the marked action of urea suggested these experiments). This salt, compared with the corresponding solutions of sodium chloride, has a specifically inhibiting action on the coagulation. The addition of 1 cc. of a 16 per cent. solution of sodium chloride frequently had a weak, though at times a somewhat stronger, inhibiting influence; 7 drops of a 16 per cent. ammonium chloride solution, or 1 cc. of a 4 per cent. solution of the same solution, had a much more pronounced inhibiting influence. One cubic centimeter of a 16 per cent. solution of acetamid had but a slightly inhibiting effect. The addition of a few drops of ammonium prevented coagulation entirely; this, however, was probably merely an alkali-action.

If the mixtures of blood serum with the above substances are kept for 24 hours in a warm room, putrefaction of the liquid usually takes place. This putrefaction does not prevent coagulation although it may inhibit it somewhat.

After the second, gelatinous, coagulation has taken place, it possible to separate a second serum from this coagulum. This second serum, to which fresh shreds of fibrin have been added, no longer coagulates.

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If, instead of the shreds of the first coagulation, the gelatinous coagulum of the second is added to the ordinary serum, efficiency of the latter fibrin is usually found not to be as pronounced as that of the first fibrin.

Foreign bodies, as such, added to the serum in place of fibrin or muscle, do not tend to accelerate coagulation. In no case could any marked influence be observed. Also platinum black was without any effect.

On the surface of the blood serum, kept in open dishes, usually a film is formed. This surface film in most cases radiates from the shreds of fibrin put into the serum. If we shake the serum for one half hour in dishes with uneven surfaces, macroscopically, visible membranes of fibrin are formed.

Extract of fibrin, which was previously kept in absolute alcohol, or of fresh fibrin under addition of chloroform water, have so far not shown an efficiency comparable to that obtained by the addition of fresh fibrin itself. One cubic centimeter of such an extract added to 4 cc. of serum, in most cases only caused a very insignificant acceleration of the coagulation in comparison to serum to which 1 cc. of a 6 per cent. sodium chloride solution had been added. Halliburton states that an extract of mammalian blood precipitated in alcohol and afterwards dried, produced coagulation in blood which had been collected in a magnesium sulphate solution. I did not succeed in producing coagulation in this way ; such a result would be contradictory to the one obtained by myself with the fibrin of rabbits and rats.

We may explain all these facts by the supposition that in the shreds produced by the first coagulation there is present a ferment or pro-ferment producing coagulation, and, further, that such a ferment or pro-ferment must be present inside of the blood cells, because the first clot consists mainly of blood corpuscles and parts of these cells. Further, we are certain of the decidedly localized action of this ferment, as directly around the shreds of fibrin the second gelatinous fibrin is deposited. This can be explained by the assumption that the ferment can only slowly diffuse into the fluid ; it must however nevertheless be able to diffuse through the gelatinous substance formed around the shreds, although as recent experiments seem to prove, even

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soap mixed with gelatine is unable to diffuse through the gelatine.

The local apposition of the gelatinous mass around the fibrin shreds produces a certain resemblance between the process of clotting and that of crystallization when caused by a crystal in a solution. If we make use of the hypothesis of the action of a ferment, we have to suppose, according to the results obtained by heating the serum, that also in the serum a small quantity of this ferment or pro-ferment is present; further, that the ferment is partially destroyed by heating it up to 46–47° C. and that it is almost entirely destroyed by heating it up to 50–51° C. There is a quantitative relation present, the coagulation taking place the more rapidly the more fibrin is added to the serum. Although absolute alcohol is used to obtain mammalian fibrin ferment, and also, according to Halliburton, that of arthropods, in these experiments absolute alcohol destroyed efficiency of arthropod fibrin ferment in a very short time.

We saw that different kinds of cells and cell products of the same species of animals had the same accelerating effect on coagulation, that however the corresponding cells of other animals were without effect. The lack of specificity in the one case combined with the specificity with regard to the species of animals used, is similar to the specificity and lack of specificity respectively found in the precipitin reactions obtained after the injection of albuminous substances in different animals.

Delezenne found that contact with the muscle causes a coagulation of the blood of birds. If the contact with muscle is avoided, it is possible to keep the blood of birds liquid for a long time, although the white blood corpuscles are still present. Lobster blood, however, in contact with the blood corpuscles, does coagulate just as quickly as if in contact with muscle. These observations, notwithstanding the presence of some minor differences, seem to point out that in these phenomena we have to deal with facts of a general significance.

III. The Conditions Under Which the First Coagulation Can be Inhibited or Entirely Prevented.

The conditions under which the first coagulation can be prevented are different from those described above for the second

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coagulation. The following experiments were mainly made with adult Limuli:

(*a*) If one collects blood at once into saturated solutions of different salts, surpassing in quantity considerably the quantity of blood received, coagulation can be to a great extent inhibited or prevented. Magnesium sulphate, sodium chloride, potassium chloride, sodium nitrate, aluminium, ammonium sulphate, potassium oxalate, sodium citrate, potassium and sodium tartrate, potassium sulphate and sodium sulphate, all have a decidedly inhibiting effect, and to a less degree sodium carbonate.

If the solutions are diluted with one half or an equal amount of water, the effect shows a corresponding decrease. Potassium salts are less effective than sodium salts; the potassium nitrate is almost without effect; the oxalates do not have any markedly stronger effect than the other saturated salts; it is therefore improbable that oxalates act in this case as precipitants of calcium, but rather as saturated salts. The blood corpuscles are in the main better preserved in these solutions. In the sodium and potassium sulphates they become roundish and send out processes after some time. In sodium nitrate and potassium and sodium chloride they retain on the whole an oval shape. Sabattani's hypothesis, that the inhibiting effect of salts is based on their action on calcium, preventing it from being ionized, becomes improbable, at least in this case. If the saturated solutions of the salts containing the blood are afterwards diluted with distilled water, many cell granules which had previously been preserved, now disappear. Many cells, however, seem to remain unchanged.

(*b*) Pyrrhol, resorcin and especially hydrochinon, have some inhibiting action on coagulation without preventing it entirely. Blood corpuscles remain more or less preserved in these solutions.

(*c*) Solutions of adrenalin chloride, 1–300, pilocarpine 1–300, and atropine 1–300, have a slightly inhibiting effect on coagulation, especially adrenalin. The cells here swell up and frequently arrange themselves through agglutination in epithelial-like order. Many cells, mainly at the margins where the adrenalin comes in contact with the blood, remain isolated or form only small clumps. The formation of a continuous coagulum however, at the margin does not take place frequently.

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(*d*) A 3 per cent. solution of formalin injected into lobsters or spider-crabs has, if the blood is shed soon afterwards, a more or less inhibiting effect on the coagulation, especially if the blood is then also collected in a 3 per cent. formalin solution.

Under such conditions it can be observed that all blood corpuscles of the lobster blood have granules, and that cells corresponding to lymphocytes are normally not present in the blood of the lobster. Their presence has lately been maintained.[1]

(*e*) The first coagulation of the blood can be prevented by previously heating the animals; the heat necessary to produce this is different in different species. The blood of small Limuli heated for 30–40 minutes up to 50–54° C. does not usually coagulate after having been shed. Lobster or *Libinia*, heated to 45–48° C. for 30 minutes loses its coagulability. Correspondingly, lobsters and spider-crabs die at a lower temperature than small Limuli. The cells usually leave the body after the animal has been heated, in a round shape and later do not usually send out pseudopodia, or do so to a less degree only. The spreading out of blood corpuscles which usually takes place under normal conditions does not take place after previous heating of the animals, or if the animal has been injected with formalin.

(*f*) Blood collected in the liver-pancreas juice does not coagulate to the same extent as ordinary blood. A large number of the blood corpuscles are usually rapidly changed into granules and many of them soon become entirely dissolved through the action of the proteolytic ferment.

(*g*) By previous injections of *Limulus* blood into turtles I have not succeeded in obtaining either precipitins for *Limulus* blood or substances which inhibit the activity of its fibrin ferment.

(*h*) *Limulus* blood collected in gelatine solutions of different concentration did not coagulate as readily as the blood collected in water, gelatine having a decidedly inhibiting action. If the solution of gelatine was very weak care had to be taken to avoid the shaking of the blood, otherwise coagulation took place. The blood corpuscles in gelatine were swollen and frequently formed epithelial-like rows.

[1] A fuller description of the blood corpuscles of *Limulus* is given in a previous paper: *Journal of Medical Research*, Vol. II., January, 1902.

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(*i*) Collected in glycerine or in chloroform the blood coagulates.

(*j*) The blood of the lobster collected in saturated urea solutions or in a 25 per cent. peptone solution, forms a gelatinous mass which does not contract in the usual way. Urea solutions have a strongly dissolving influence on the blood corpuscles. The shreds resulting from the first coagulation of the lobster blood put into urea solutions, become transparent and swell; in glycerine they also become transparent but do not swell; returned into water they regain their usual characters.

IV. On the Influence of Mechanical Agencies on the Protoplasm of the Blood Cells of Some Arthropods.

It has been observed by previous investigators that the blood cells may send out long processes which can adhere to solid particles. It is however possible to produce a much greater change. The protoplasm of a little clump of blood cells can be transformed into a system of threads of different sizes. It is best to use for such experiments cells which have been well preserved and are somewhat swollen; it is also necessary to use blood in which it is possible to exclude the presence of an extra-cellular mass of fibrin. Such cells are found, *e. g.*, in blood collected in gelatine solutions or in adrenalin or, also, in hydrochinon.

The necessary traction can be applied either by putting a second slide on the first containing the blood in solution and separating the two slides after they have been pressed together or by whipping with a needle the blood cells on the slide. The whole process can be followed under the microscope; one can see how cells arranged in a row become transformed into a system of threads which no longer indicates that it is derived from cells. Sometimes however we can see in the center of such threads the nuclei of the cells, adapting themselves to the thickness of the thread, or they may bulge out of the center of the fiber as little spheres. Under such circumstances one single cell can be changed into a very long thread. It can also be seen that frequently spindle-shaped cells arrange themselves into

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a single row and may form one long continuous thread passing over a large part of the slide.

Sometimes very little effort is needed to achieve such a transformation. If we move the solution containing a few drops of blood in one direction the flow of the liquid may be sufficient to arrange the cells into rows and draw them out into continuous, long threads.

Such fibrils are, however, not only formed from the protoplasm of the entire cell but they also may be formed inside of cells. This can be observed, for instance, if we exert traction by a needle in a network of cells spread out on the slide, *e. g.*, about half an hour after the blood has been shed. If we merely exert pressure on masses of cells we may change them into a structureless gelatinous mass. By exerting afterwards traction on such a gelatinous mass, using two slides, we can change this mass into a system of fibrils of different diameters; such fibrils can again be rolled up into a gelatinous mass. The protoplasm has in this case the consistency of a dough, differing however in that it is somewhat more elastic.

If we apply these mechanical agencies to cells immediately after they have left the body, we find the protoplasm more brittle and the cells can under these conditions by pressure easily be separated into small granular particles.

A transformation into fibrils can also be produced from the protoplasm which had previously left the body of the ruptured cell, or from the protoplasm of exploded cells. The same agencies, traction and pressure, are also able to make the granules of the cells disappear. Cells which had been previously kept for a longer period in saturated salt solutions or in formalin, no longer yielded such a fibrillar mass.

The physical properties of this fibrin-like mass are very similar to the fibrin formed through the coagulation of the extra-cellular part of the blood. The cellular fibrin can be drawn out, and as it is somewhat elastic, may afterwards slowly retract. It is also sticky. The cells themselves have similar physical properties. If they are spread out, as is usually the case, one half to one hour after coagulation they are kept under great tension, and if liberated from the surface of the slide by a needle they retract.

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They also are sticky. A cell, hanging on a fiber after coagulation of the blood has taken place, sticks to the surface of the slide; after having sent out processes, if as a result of outside motion a pseudopodium of the cell touches the surface of the glass.

Very similar fibrils can be produced if we subject the blood serum of *Limulus* or lobster to the same mechanical agencies. It usually however needs more exertion to obtain the same result from these colloidal solutions than from the cells.

V.

Certain analogies may be pointed out which exist between certain processes during coagulation of the blood and certain other phenomena.

(*a*) Inside of the body blood corpuscles have an oval shape and do not send out pseudopodia. After having left the body certain changes in the environment take place, the blood cells send out pseudopodia, and somewhat later they begin to spread out over the whole surface of the slide, forming one continuous network. We see a certain analogy between this process and the emigration of leucocytes under so-called inflammatory conditions.

The changes which lead to the spreading out of the blood corpuscles of arthropods cannot, under the given conditions, be determined by chemotropic influences acting from certain directions. The chemical conditions on the slide are the same on all sides; nevertheless, the cells move and spread out. Inflammatory conditions in the higher animals also mean a change of the normal environment brought by toxic or other causes. Thus it may be that the leucocytes of vertebrates do not migrate primarily under the influence of chemotactically acting substances, but under the influence of certain physico-chemical factors which bring about changes in the state of the protoplasm of these cells; direct chemical stimulants may be added secondarily.

(*b*) During regenerative processes of the epithelium movements of the cells take place independent of preceding cell multiplication. The epithelial cells under these conditions usually move in contact with solid bodies, parts of which, as I have previously shown, may be taken into the cell body itself. It can be observed under

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these conditions that the protoplasm of the epithelium is in very close connection with the solid body, for instance the coagulum forming a scab, and if one raises the scab from the wound parts of the epithelium may remain in contact with it and are raised with it. It is not unlikely that similar changes in the consistency of the protoplasm of the epithelial cells take place during regeneration, as take place in the blood cells after they have left the body, and the adhesion may in both cases have to be explained by a change in the consistency of the protoplasm which makes the cell or part of it sticky.

(*c*) We have been able to show that under the influence of traction the protoplasm of the cells assumes a fibrillar structure and that these fibrils frequently are continuous through a number of cells. Similar phenomena can be observed in many other cells. Epithelial fibrils, for instance, are not limited to one cell and especially under the influence of traction exerted during the regenerative process, the fibrils passing through more than one cell are drawn in the direction of the traction exerted and indicate therefore the movements of the epithelium. In a similar way we find fibrils passing through many cells, for instance in the heart muscle, in the developing white fibrous tissue and neuroglia and probably also in the peripheral nerves and in ganglia cells. The same mechanical factor bringing about fibrillation which can be observed experimentally in the protoplasm of blood cells, may be of importance also in these cases. The main factor is of course the specific structure of the different cells determining the specific structure of these fibrils.

(*d*) The blood corpuscles which in the body under normal conditions do not change their oval form, produce outside the body an agglomeration of cells which may be compared to a tissue. Under certain conditions, *e. g.*, in gelatine or in adrenalin, or even in distilled water, many cells form through agglutination an epithelial-like tissue; under ordinary conditions however the cells soon form intercellular substances and the appearance of connective tissue is produced. The network of spread-out cells closely resembles the reticular tissue. The exoplasm of the spread-out cells under the influence of slight mechanical agitation, takes on a fibrillar character. If we make sections through a coagulum in

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which the blood corpuscles have spread out, the appearance of connective tissue is presented. We see apparently intact cells surrounded by fibrils. These cells, however, only represent the endoplasmodic part of the original blood corpuscles, the exoplasmatic part having been transformed into fibrils. Many of these fibrils stain very well by Mallory's connective tissue stain.

In a similar way, the fibers of fibrous tissue and of neuroglia in mammalians seem to be produced from the exoplasm of the cells. In coagulating blood, however, whole cells may be changed into fibers. Here protoplasm having left the cell body and being now entirely intercellular may also form fibrils. Such an occurrence can easily be observed under the microscope in the blood, these changes taking place in a short time. It would be more difficult to prove such an occurrence in the development of connective tissue. The morphological similarity (not identity) between the processes in the blood cells and connective-tissue cells being apparently so great, it ought to be considered, whether an intercellular origin of fibers from protoplasm, secondarily disconnected from cells may not also take place in connective tissue.

We can observe that the granules of the blood cells frequently spread out and assume in the end an intercellular situation, and that they not infrequently become included in fibrils. Similar observations have been made in the developing cartilage for instance.

As we have seen, the formation of intercellular substances in the coagulating blood can be prevented if we collect the blood cells in certain solutions, which inhibit that further changes in the cells take place without preventing the possibility of such changes, if the solution is changed afterwards. We may therefore say that one of the conditions under which the formation of an intercellular substance takes place is the presence of a certain fluid which surrounds the blood cells which have left the body. The serum has therefore a more or less cytolytic power and the formation of "connective tissue" is based on a partial destruction or dissolution (cytolysis) of the blood cells by which protoplasm leaving the cells becomes changed into intercellular fibrils.

Summary.

1. The following factors play a part in the coagulation of the blood of arthropods:

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(*a*) Agglutination of blood cells.

(*b*) The formation of a gelatinous mass, and secondarily the formation of fibrils from protoplasm exuded from cells and from protoplasm of cells entirely dissolved.

(*c*) The spreading out of the blood cells during the next few hours after shedding of the blood.

(*d*) The coagulation of a fibrinogenous substance.

2. The agglomeration of the blood cells leads to the formation of tissue-like structures. The formation of the fibrillar intercellular substance corresponds to the formation of fibrillar connective tissue. By changing the cytolytic medium in which the cells are suspended, the formation of an intercellular fibrillar substance can be prevented. By agglutination of cells which remain well preserved, an epithelial arrangement of the blood cells can be brought about.

3. Inside of the normal body the blood cells do not change their form; this however takes place outside of the body, without the presence of certain specifically directing chemotropic stimuli. The cell protoplasm moves in contact with the solid surface to which it sticks. In a similar way the leucocytes react during an inflammatory process and a certain analogy also exists between this process and the movement of the epithelium during regeneration.

4. Coagulation of the blood can be prevented or inhibited by receiving it in different solutions, such solutions usually having also a preservative influence upon the cells. Oxalates act in a similar way to other salt solutions, that is if they are very concentrated. This points to the fact that in all probability the precipitation of calcium is, under these conditions, not the essential factor. Sodium and potassium sulphate have, in a similar way as sodium nitrate and sodium and potassium chloride, an inhibiting influence upon coagulation. The blood cells themselves, however, are influenced in a different way by the two sulphates and the other salts.

5. The coagulation of the blood is inhibited by receiving the blood in gelatine solutions; collecting it in oil is without influence.

6. The second coagulation of the lobster blood is inhibited by means different from those acting on the first coagulation. The

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facts observed during the second coagulation can be explained through the hypothesis of the action of a fibrin ferment present in the blood cells and in the muscle of the lobster, which however is not present in the blood cells or in the muscle of certain vertebrates. This points to a multiplicity of fibrin ferments in different species of animals and points to the identity of the ferment in different tissues of the same species of animals. This specificity in the one case and the want of it in the other case corresponds to a similar specificity and want of specificity as has been found to exist with the precipitins obtained through injection of certain body fluids or albuminous substances into different species of animals.

7. Potassium cyanide, urea and solutions of peptone inhibit the second coagulation. If these substances are add to the serum their inhibiting influence is in a reverse proportion to thire strength if they act directly upon the fibrin which contains the ferment. Potassium cyanide has the strongest, peptone the weakest action upon the fibrin, and conversely, peptone has the strongest and potassium cyanide the weakest action on being mixed with serum.

8. The necessity of the presence of calcium for the second coagulation can be easily proved. Ammonium chloride has a stronger inhibiting action on the second coagulation than sodium chloride.

9. Mechanical agencies, namely, pressure and traction, change the cell protoplasm into a system of fibers which cannot be distinguished in its physical properties and appearances from the extra-cellular fibers. The cell granules disappear under the influence of traction and pressure, just as the granules disappear spontaneously in the cells during coagulation of the blood. By traction and pressure exerted upon the blood serum free from cells, similar fibrillar structures can be produced as in the protoplasm of the cell. The fibrillar structure of the protoplasm seems therefore to be a secondary condition, produced by mechanical influence upon albuminous substances which may be common to the protoplasm of the cell and to solutions of certain albuminous substances.

10. By pressure and traction the cell protoplasm can be changed into fibers whose arrangement is determined by the


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direction in which the mechanical forces act; mechanical factors may also determine the direction of the fibrils in connective tissue and bone.

11. The significance of the blood corpuscles for the coagulation of the blood consists therefore in the following: (*i*) The cells themselves are transformed into substances similar to fibrin, (*ii*) They accelerate a coagulation taking place in the surrounding fluid; some facts point to the possibility that the coagulating substance contained in the serum has its origin in the blood cells.

12. Towards foreign substances introduced into the body of the animal, the blood corpuscles of *Limulus* do not behave as actively as the blood corpuscles of mammals. An active penetration into these foreign substances cannot be observed in *Limulus*, the cells only take part in the formation of the coagulum around the foreign substance.

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THE PALÆOCHEMISTRY OF THE OCEAN IN RELATION TO ANIMAL AND VEGETABLE PROTOPLASM

BY

A. B. MACALLUM, M.A., M.B., Ph.D.

Professor of Physiology in the University of Toronto

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I.—INTRODUCTION

THE history of the composition of ocean water is a question of very great interest to the geologist, the physiographer and the biologist. To the geologist and physiographer its importance lies chiefly in the fact that it is associated with the history, on the one hand, of erosion and denudation of land surfaces of the globe, and, on the other, of the formation of all the sedimentary strata. The ocean, ever since the first condensation of water on the rockcrust of the earth, has acted as a gigantic solvent, and the salts it now holds in solution represent what it has retained after its action for millions of years as a leaching and filtering agent. The sedimentary rocks are thus but a vast precipitate from the ocean of what had been partly suspended and partly dissolved matter in it during all the geological periods. The history of the composition of the ocean is, on this view, the complement of the history of all the terrigenous changes necessary to fill out all the pages of the record of events that have transformed the surface of the earth.

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To the biologist the value of the question obtains from a different point of view. The sea is the original home of all life on the globe, and it was in the sea that the differentiation between animal and vegetable life, as well as the evolution of the great divisions of the animal kingdom were effected. Indeed the great events in the evolution of animal forms have been rendered possible by changes which have taken place in the composition of ocean water. These changes have modified organisms, and have created conditions which have served as factors in directing the course of development. This may be specially illustrated by reference to the case of the calcium salts in sea water. That the earlier Archæan seas contained comparatively small quantities of calcium compounds seems to be clearly indicated by the fact that in pre-Cambrian strata the limestone deposits are very limited, not more than two per cent. of the thickness of the beds, the Huronian portions of which, now generally recognized as of sedimentary origin, are, according to Lawson,* over 50,000 feet in thickness. The small amount of limestone deposits could not have been due to the absence of living organisms, for the oldest Cambrian beds contain Trilobites and Brachiopods, and such highly specialized forms postulate a long course of pre-Cambrian life. The very fact that the Brachiopods of the early Cambrian were largely those provided with a horny or chitinous shell, indicates that all the animal forms of the preceding period had imperfectly acquired the lime "habit," which, one may reasonably believe, would have earlier made its appearance had calcium salts been present in considerable quantities in ocean water from the first. It is perhaps due to the absence of this lime "habit" that fossils do not obtain in pre-Cambrian strata.

Once, however, the lime "habit" was acquired, through adaptation of the animal cell to its environment, the course of development became accelerated, and the evolution of the higher types of Invertebrate life, as well as all the forms of Vertebrata, became possible. The Vertebrate skeleton, and all that it implies in evolution, is, therefore, a result of the gradual increase in the quantity of calcium in the oceans of the pre-Cambrian period.

To both the geologist and the biologist the history of the chemistry of the ocean has recently acquired an additional interest from the attempt made by Joly† to determine the age of the earth, who uses for that purpose as factors the amount of sodium now in the ocean, and that

* Geol. Survey of Canada, 1887, pp. 101 and 102, F.

† An Estimate of the Geological Age of the Earth. Trans. Roy. Dublin Soc., Vol. 7. (Ser. 2), 899, p. 23.

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estimated to be in the annual river discharge of the globe. Joly took for these the results of Murray,* who, basing his calculations on the discharge of nineteen of the principal rivers of the world, estimated the total amount of the sodium and other salts annually put into the sea by river water. Joly finds from Murray's tables that the sodium annually discharged is 157,270,000 tons, and the quantity in the sea is 14,151,000,000,000,000 tons. Dividing the latter by the former he gets as quotient, approximately, 90,000,000, which, expressed as years, would be the age of the earth, or, rather, the period of time which has elapsed since the first condensation of water vapour took place on the globe. Joly admits that the ocean at first contained a considerable quantity of sodium as sodium chloride, and this he puts at about 14 per cent. of the present amount in the sea. This would make the amount discharged into the sea by river water less than that stated above, but, on the other hand, the volume of the ocean may, as a result of more recent estimations, be given a higher value, and in consequence the mass of sodium in it would be 15,627,000,000,000,000 tons. Further, of the sodium annually put into the ocean, Joly allows as much as 10 per cent. for that which is taken from the ocean by the rain and returned again in river water, and this estimate would make the amount of river sodium, which is annually leached out of the rocks and strata, as 97,800,000 tons. With these values Joly finds that the corrected figures for the age of the earth is 89,300,000 years.

In support of his contention Joly shows that as compared with the igneous rocks there is in the sedimentary rocks, which are derived from them, a deficiency of sodium, and that the sodium now in the sea would approximately account for the difference. The bearing of this fact is that all the sodium now in the ocean was derived from the original rock crust by processes which to-day are in operation in decomposing rock material and removing the sodium therefrom. In other words, the discharge of sodium into the sea has been in the past a uniform one, or at least subject to no great variations that would constitute a factor against determining the age of the earth by this method.

This estimate has been ably criticized by the eminent geologist, the Rev. Osmond Fisher,† who points out that the sodium which is derived from the decomposition of crystalline or igneous rocks is in the form of carbonate rather than chloride; and he asks whether it is not possible that the chloride of river water is derived, not from crystalline,

* On the Total Annual Rainfall on the Land of the Globe, and the Relation of Rainfall to the Annual Discharge of Rivers. The Scottish Geogr. Mag., Vol. 3, 1887, p. 65.

† Geol. Mag., New Ser., Vol. 7, p. 124, 1900.

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but from sedimentary rocks, or from what Sterry Hunt calls "fossil sea water, still to be found imprisoned in the pores of the older stratified rocks, and presumably in the younger as well." To answer this affirmatively would be of necessity to assert that the sodium which now goes to the sea as sodium chloride comes from the supply derived from and deposited by the sea in ancient geological strata—that is, what was at one time in the sea is being returned to it again. Fisher also points out that the strata which are now in the process of formation, imprison sodium chloride in their mass, taking it from the sea. There would thus be a constant circulation of sodium chloride from the ocean to the stratified rocks and back again to the ocean. That would also postulate that the sea was almost as rich in sodium chloride in Silurian times as it is now, and it would go far to support the view that "the sea was salt from the first;" but if we assume that the sodium of the sea is derived from those sodium compounds supplied by rivers other than the chloride, the estimate of the age of the earth, as given by Joly, would have to be multiplied several times in order to get the approximate length of the period which has elapsed since the oceans of the globe were first formed.

Another criticism of Joly's view, made along the lines followed by Fisher, is that advanced by Dubois,* who, from a comparison of the amounts of sodium and chlorine supplied to the sea by a large number of rivers, concluded that only a small portion, if any at all, of the sodium derived from denudation appears in river water as sodium chloride; that the sodium chloride discharged into the sea annually is derived from the rainfall, and the salt deposited in the older strata by the sea.

As Fisher has already pointed out, it is the sodium compounds other than the chloride that ought to be considered as being primarily derived from the disintegration of rock mass, and, therefore, primarily added to the sea. What the total amount of this sodium is cannot be determined with approximate certainty, but Dubois is inclined to regard it as about one quarter of the total discharge of sodium into the sea as given in Murray's tables, and, consequently, Joly's estimate of the length of the period which has elapsed since water first condensed on the earth's surface would have to be multiplied by four, the product being approximately 400 million years.

* On the Supply of Sodium and Chlorine by the Rivers to the Sea. Kon. Akad. v. Wetensch., Amsterdam, Proceedings of the Section of Sciences, Vol. 4, p. 388, 1902.

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II.—THE ORIGIN OF THE PHYSIOLOGICAL RELATION OF THE CHEMICAL ELEMENT IN BLOOD PLASMA.

I have thus dealt at some length upon the importance of the history of sea water, and with Joly's views and those of his critics, because all this leads up to a question which is of very great importance to the physiologist. The life of the globe in the earlier geological ages, so far as the strata reveal to us the past history of the earth, as already pointed out, was closely associated with the sea. It is indeed almost universally assumed that life began in the ocean and continued in association with it alone till the close of the Cambrian period, although the presence of graphite in Cambrian and older rocks seems to indicate that vegetable organisms were accommodating themselves to a land life. Even this may not be an exception, for these rocks must have been laid down under water, and therefore their organic remains would be those of the sea. If accordingly we could know what the composition of the sea water in the Cambrian and pre-Cambrian periods was, we would, in all probability, be able to determine some of the chemical and physical forces to which living matter was then subjected and thus explain the relations which obtain to-day in living matter between it and its salts. In a recent paper* I have pointed out that the relative proportions of the elements, sodium, potassium, and calcium in the plasma of the blood are surprisingly very like those which are found in the ocean water of to-day, and that the differences which obtain between the two series of proportions of these elements may be explained on the ground that such proportions in the blood plasma are those that obtained in ancient sea water when the ancestral form of Vertebrates, in which sea water was the circulatory fluid, as it is in many marine forms to-day, acquired a closed circulatory system. That the ancient proportions are reproduced to-day in all forms, which have a closed circulation, I attribute to the influence of heredity, the cells of the organisms having for ages been associated with the sodium, potassium, and calcium in certain proportions, and having been accommodated to them, the relations ultimately became so fixed that living matter reproduces the ancient proportions in the fluids which bathe itself. There is one point in which the proportions in the circulatory fluid and those in sea water differ, and that is in respect to the magnesium. In the sea water of to-day there are 11.99 parts of magnesium for every 100 of sodium, while in plasma there are 0.8 parts of magnesium to 100 of sodium. This is

* On the Inorganic Composition of the Medusæ, Aurelia flavidula and Cyanea Arctica. Journ. of Physiol., Vol. 29, p. 213, 1903.

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a striking difference but it is easy of explanation. The proportion of magnesium in sea water is now slowly growing. In the pre-Cambrian oceans it must, therefore, have been very small, not perhaps as low as it is in blood plasma, for in the latter the magnesium would only represent the proportion of an earlier period than that in which the circulation became closed, as the tissues would only reproduce the proportion which had by long accommodation become fixed in them. Even the organisms which live in the sea to-day, whose ancestral forms have lived in the sea since the Cambrian, do not take up the magnesium from the sea water in the full proportion which it has in the latter.

III.—The Origin of the Relation of the Chemical Elements within Protoplasm Itself.

There is, therefore, so far as the circulatory fluid of Vertebrates is concerned, a reproduction of the proportions of the sodium, calcium, and potassium of the pre-Cambrian oceans. The problem which now arises is one whose solution involves greater difficulties. If organisms should reproduce in their own circulatory fluids the proportions of the elements in the early geological periods, what contributed to those remarkable proportions which obtain, not in the circulatory fluids, but in the living matter itself? These proportions are widely different from those found in the circulatory fluids, and one cannot bring oneself to regard the former as derived from the latter. In vegetable organisms the potassium and the calcium much exceed the sodium, and even the magnesium may be greater in amount than that of the latter. In animal organisms the proportions are difficult to ascertain owing to the presence of skeletal and other structures in which the calcium and sodium greatly preponderate, but even in these the potassium is nearly equal to the sodium, and in muscle it is greatly in excess, while the calcium and the magnesium are much less than the sodium. Thus, in the muscle of the dog the relative values for each are* :—

Na.	*K.*	*Ca.*	*Mg.*
100	354	7.26	25.1

These proportions may or may not represent approximately those found in unicellular organisms like an Amœba, or even a white blood corpuscle, but do they represent to any degree the proportions which obtained in the early pre-Cambrian seas when life was represented by unicellular organisms only, which accommodated themselves to the sodium, potassium, calcium, and magnesium in their habitat, just as the

* Julius Katz, Pflüger's Arch., Vol. 53, p. 1, 1896.

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marine unicellular organisms of to-day have accommodated themselves to these elements in the sea water? If the blood plasma of Vertebrates, because of the forces of heredity, reproduce the proportions which obtained in pre-Cambrian oceans, why should not the cells of the tissues because of the same forces, reproduce in themselves the proportions which obtained in sea water of a much earlier geological period? In other words, if the proportions in the plasma are inherited, why should not those found in the living matter be considered as inherited also? An affirmative answer to this question would postulate that the proportions of the four elements in early pre-Cambrian seas were very greatly different from what they are now in the ocean—as different almost as the proportions of the four elements in muscle are from those found in the blood plasma.

The question is one of great importance in physiology, and, though its solution presents great difficulties, its very interest compels a consideration of it. We know that the unit of living matter, the cell, whether of animal or vegetable kingdom, presents, on the whole, the same type of structure, and it goes through the same morphological changes. Some of these are grouped under the process of division, and its characteristic details are the same in both animal and vegetable forms. Now, the animal and vegetable cells are derived from a single type which must have existed at the very dawn of life on the globe. The whole process of division, with its peculiar morphological features, was elaborated in this single-celled organism, which transmitted it to its descendants. Since, as already stated, the process of division is the same in both kingdoms, it is obvious that it has continued almost unchanged through an infinity of generations, animal and vegetable, and for many millions of years, and that this preservation of the original type is due to heredity. If, now, heredity is so powerful in regard to structure, is it a negligible force in regard to chemical composition? Is living matter fixed in structure almost beyond change, however widely the conditions under which it lives may vary, but unfixed and changeable in its relations to the chemical elements? As structure depends so largely on composition, it would be difficult to explain how living matter could so widely vary its relations to the elements and at the same time retain its structure.

We are, therefore, forced to a choice of hypotheses of which one postulates that all of the relations of living matter to sodium, potassium, calcium and magnesium are a result of inherited forces, while the other concedes that in regard to the circulatory fluids the proportions are

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determined by heredity, but the relations of these elements in living matter itself are due to quite different forces in which heredity is a small factor or no factor at all. The acceptance or rejection of either hypothesis depends on the evidence which we can bring as to the composition of the ocean in the very earliest geological periods.

The conclusions which we can formulate on this point depend on what we accept as the composition of the original crust of the lithosphere, and in our knowledge of the character and composition of the sedimentary rocks, and they must also be based on the changes which are admitted to have taken place in the composition of the ocean during all the periods. These conclusions I propose to deal with here in a general way only, for a full consideration of all the facts which have a bearing on them would demand a detailed treatment which would far exceed the limits set for this paper.

IV.—The Composition of the Primeval Ocean.

The original condition of the earth was a molten mass in which the temperature was so high that many of the elements now in the rock crust were in a gaseous condition, and dissociated, just as they are at present, in the solar atmosphere. As the dissipation of heat went on some of these must have condensed at degrees of temperature which approximated their present respective volatilization points, while the remainder, oxygen, hydrogen, chlorine, sulphur and carbon would combine to form water, hydrochloric, sulphuric and carbonic acids. The elements, sodium, potassium, calcium, magnesium, and aluminum would also before condensation take out of the original atmosphere chlorine, sulphuric acid, oxygen, and perhaps, carbonic acid, to form the chlorides, sulphates, oxides, and carbonates of these elements, but whether these compounds obtained after condensation depended on whether the temperature of the heated rock surface was still as high as their respective dissociation points. When the molten magma had cooled down to a degree below the lowest dissociation point, all the compounds referred to would be either deposited on the hot rock surface or in the form of vapour in the then atmosphere. When the temperature of the latter had fallen to about 1000°C, all these compounds were removed by condensation, for although, under the atmospheric pressure which now obtains, the temperature of condensation is for nearly all these compounds about 200° lower, the very great atmospheric pressure of the pre-oceanic period must have rendered the

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combination of the dissociated elements and the condensation of the compounds formed from them possible at a much higher temperature.*

At such a temperature the previously molten rock had become rigid, and of course the condensed compounds would be deposited on its surface, and when refusion of the rockcrust occurred, as it must have done over large areas, large quantities of the deposited compounds would be diffused through the superficial crust. When the cooling of the atmosphere and globe progressed until the temperature of the former was 370°C, the first condensation of water took place on the rock surface. The atmospheric pressure, according to Joly,† must have been about 270 times what it is now. According to Clarke's‡ estimate of the relative values of water and carbon dioxide to that of the solid portion of the globe, the atmospheric pressure before the first condensation took place, was about 247 times what it is at present. Joly affirms that at 370°C a pressure of 190 atmospheres would produce a condensation of water, and, as the pressure was much higher, condensation would go on till the pressure fell below 190 atmospheres. This would entail rapid evaporation, for at many points the temperature of the rock surface would be so high that the water would condense only to boil away immediately. This would collect the salts deposited on the surface in masses, and it would, as in the case of the chlorides of magnesium, iron and aluminium, convert these into oxides of these metals and free chlorine, which, uniting with hydrogen, would form free hydrochloric acid. The other chlorides, namely, those of sodium, potassium and calcium would be unaffected. The ferric chloride would in some cases be volatilized but to be recondensed.

This condensation of the water vapour, and the re-evaporation would occur a countless number of times before there would obtain a permanent body of water on the globe. Where such first occurred there would be a lower temperature than elsewhere, and in consequence further condensation of water vapour would occur there also. The result would be the first ocean basin, the weight of the body of water acting on the

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*The volatilization points of potassium, sodium and magesium are 667°C, 742°C, and 1100°C respectively. The melting points of calcium and aluminium are unknown. The melting points of certain sodium and potassium compounds are, according to V. Meyer & Riddle (Ber. d. d. Chem. Gesell. Vol. 27, p. 2,443,) as follows:

Na Cl	851°C.	K Cl	766°C.
Na Br	727°C.	K Br	715°C.
Na I	650°C.	K I	623°C.
Na_2CO_3	1098°C.	K_2CO_3	1045°C.
Na_2SO_4	843°C.	K_2SO_4	1073°C.

† *Op. cit.*

‡ F. W. Clarke, The Relative Abundance of the Chemical Elements. Bulletin U. S. Geol. Survey No. 78, 1891.

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thin crust and easily affecting the depression. These phenomena would be repeated at other points as the temperature of the crust and the atmosphere gradually lowered, until at a point below 100°C. nearly all of the water originally present in the atmosphere had condensed to form the oceans of the globe.

The composition of the ocean would follow from the occurrence of the soluble chlorides, sulphates and carbonates of the metals which came in contact with the first condensations. As pointed out, the condensation of superheated water would convert the chlorides of magnesium, iron and aluminium into magnesia (Mg O) oxide of iron ($Fe_2 O_3$) and alumina ($Al_2 O_3$), the first of which is soluble only in 55368 parts of hot or cold water,* while the two latter are practically insoluble, even in dilute acids. The magnesia, of course, would dissolve in water which contained either hydrochloric or carbonic acids, but the amount dissolved would, on account of the slight quantity of these acids in the water, be very small. The other chlorides, namely, those of sodium, potassium and calcium, although equally abundant, would not be leached out of the rock surface in equal amounts. The solubilities of these salts differ. For example, 100 parts of water dissolve at 99°C 154 parts of calcium chloride, 56.3 parts of potassium chloride, but only 39.7 parts of sodium chloride. In consequence there would be different quantities of each chloride dissolved, and the calcium chloride would by far predominate, while the potassium chloride would be more abundant than the corresponding sodium compound. There would, as already pointed out, be very little ferric chloride and what would be dissolved would gradually all be converted, first into the colloidal ferric hydrate, and eventually into the insoluble oxide of iron.

It does not follow that the ocean would contain, even after a long period of action on the rockcrust, the whole of the chlorides of calcium, potassium and sodium originally disposed over and diffused through the now more or less rigid rockcrust. The constant washing out of the land areas would no doubt tend to remove these salts from the rocks until there would be little left in the latter and at the same time they would become correspondingly more abundant in the sea water. But other salts would begin to appear there also. The magnesia derived from the chloride of magnesium, through the action of superheated water, would, under the action of carbonic acid in the rain water go into solution as carbonate, but the amount so dissolved, would, on account of its low degree of solubility, be very small and it would only

*Fresenius, Liebig's Annalen, Vol. 59, p. 121.

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after a long period of time become appreciable in the ocean. The carbonic acid in the rain water must have acted, as it does now, on the silicates of sodium, potassium and calcium in the rocks and produced free silica and carbonates of these elements, these latter going into solution and thus reaching the ocean, where, acting on the chloride of calcium, carbonate of lime and chloride of sodium and potassium would be formed. The calcium carbonates would be removed by deposition and thus constitute the origin of the limestone beds of the pre-Cambrian age, but the chlorides remaining in solution, thus contributed to an increase in the amount of sodium and potassium in the sea water.*

The sulphates in the rock crust disintegrated or affected would also be carried to the sea, but, as these would be small in quantity, they need not be specially considered here.

Thus the history of the sea must have begun and continued for a period of unknown length. The only change came from the discharge into the sea of the carbonates, the consequent removal of the lime and the slow increase in amount of magnesium, sulphuric acid, and of potassium and sodium. The two latter elements were not removed from the sea except through the rainfall. As I shall presently point out, the potassium compounds are to-day removed from the ocean apparently as rapidly as they are added by river water, and, in consequence, the amount in sea water now appears to be stationary. In the earliest geological period the conditions which now contribute to this result did not exist, and the ocean retained all the potassium it held or received through river discharge. In all probability the potassium equalled, and even exceeded, the sodium in amount.† When sediments began to form, and, when soils made their appearance, then, and then only began the elimination of the potassium from the ocean. It has been long established that potassium manifests a marked capacity to unite with silicates of alumina to form firm compounds, and these obtain whenever potassium salts in solution come in contact with argillaceous material, sedimentary or otherwise,‡ while the sodium, magnesium, and calcium are unaffected.

* Sterry Hunt (Chemical and Geological Essays, Boston, 1875) held the view that the most abundant constituent in primeval sea water was calcium chloride, and that with the gradual addition of sodium carbonate calcium was removed as carbonate and sodium chloride consequently took its place.

† Joly (*loc. cit.*) assumes that the greater part of the chlorine now in the ocean was originally united with the iron, calcium, magnesium, potassium, and sodium, these elements entering into combination in proportion parallel to the proportions in the rockcrust as determined by F. W. Clarke (*loc. cit.*) This postulates that 14 per cent. of the chlorine now in the ocean was united with sodium, and consequently the ocean originally contained about one-seventh of the sodium it now holds. As the proportion of sodium to potassium in the rock crust is 100 to 95, on Joly's hypothesis the potassium in the primæval ocean must have really equalled in amount the sodium therein. Joly, however, is in error in supposing that the chlorides of magnesium and iron could have existed, and he should consequently have made a greater allowance for the amounts of chlorine combined with the sodium, potassium, and calcium.

‡ Sterry Hunt (*op. cit.* p. 95.)

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The capacity to abstract the potassium is increased if the silicates are mixed with organic matter. Consequently the potassium which rain water may contain is in great part removed when the latter filters through soils, and, therefore, the water discharge from alluvial areas is always richer in sodium than potassium. This capacity of soils to abstract potassium is a matter of direct demonstration, and it "explains the presence of so small an amount of potassium salts in the waters of rivers, lakes, streams, and oceans where the lime and soda have accumulated."* This cause of deficiency acts not only in the case of the potassium leached out of disintegrating rock by rain water, but also on the potassium carried from the sea to the land areas by rain water. The potassium thus carried is not inconsiderable, for, according to M. J. Pierre,† the rain water in the neighbourhood of Caen (France) annually carries to each hectare of land, about 7.9 kilograms of this element, or about 1.23 tons per square mile.

This mode of elimination also operates in the ocean, where, however, the organic matter responsible for the removal, is derived from plankton organisms, which, on dying, fall to the sea bottom and their remains decomposing, the potassium they hold reacts with the argillaceous material on which the deposits rest and forms the mineral known as glauconite, containing as low as 0.95 per cent. of oxide of potassium, but other estimates range from 2.52 to 4.21 per cent. The sodium present is very much less in quantity.‡ This mineral is now being formed, as it has been formed in the past, on the ocean bottom over the areas which fringe the continental coasts and it constitutes as much as, or more than, half of the deposits in shallower waters. Considering the extent of these areas as well as the fact that they cover the sea bottom of those localities into which river discharge takes place, it will be recognized what a very important factor the constant formation of glauconite is in eliminating potassium from sea water and thus preventing an increase in the amount of that element in the ocean. This formation has been going on in the past geological periods, for it is to be found§ in the primary formations of Russia and Sweden, in the sands

* Mendeleef's Chemistry, Vol. 1, p. 547, 1897.

† The reference is given in Dr. Angus Smith's "Air and Rain," which is quoted by Joly (*loc. cit.*)

‡ The analysis of five specimens as given by Murray & Renaud (Challenger Report, Deep Sea Deposits, p. 389) gave:

	I.	*II.*	*III.*	*IV.*	*V.*
Ca O	1.69	1.26	1.27	1.34	1.19
Mg O	2.49	3.13	3.04	2.83	4.62
K_2O	2.52	4.21	3.86	3.36	0.95
Na_2O	0.90	0.25	0.25	0.27	0.62

Other analyses quoted by Roth, (Allgemeine and Chemische Geologie, Vol. 1, p. 559, 1879) gave a percentage of potassium (not K_2O) varying from 2.8 to 7.3.

§ Murray & Renaud, op. *cit.*, p. 384.

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and gravels of the Cambrian sandstone of North America, in the Quebec group of Canada and in the coarse Silurian sands of Bohemia. In the Mesozoic period it was more abundantly formed and its deposits are very marked in the strata of the Cretaceous division. It is also found in the Tertiary from the lowest strata to the highest of the series. It is thus shown that the formation of glauconite occurred in all the geological periods from the commencement of the Palæozoic Age to the present time and that thus a very large proportion of the potassium which the ocean would now contain, were it not for the formation of glauconite, has been removed from it.*

In the formation of glauconite, organisms appear to play a very distinct part and amongst these the Foraminifera are the most important. The decomposing organic matter of the dead forms liberates sulphur which combines with the iron in deposits to form sulphide.† This latter is converted into sulphuric acid which, acting on the fine clay sets free colloidal silica and ferric hydrate in a condition which promotes their union and the silicate so formed combines with potassium to form glauconite. It is obvious that organic matter is a very important factor in the process and that in the absence of animal organisms no glauconite would be formed, a view which explains the almost complete absence of this mineral from the deep sea areas, but it also postulates as decidedly, that before the appearance of living forms in the primeval ocean, there was little or no potassium eliminated from it, and this, taken in conjunction with the fact that in earlier pre-Cambrian times there could not have been much or any soil to affect the potassium in the waters discharged from the land areas, makes it quite clear that there was a period during which the potassium content of the ocean must have increased absolutely and that this was succeeded by a period in which the amount of the potassium ceased to increase or remained practically stationary, while decreasing relatively to other constituent elements. The beginning of this latter period coincided with the appearance of living forms in large numbers in the sea.

The history of sodium in the ocean has been one of uniform increase through all the geological ages. The addition that is to-day being made by river discharge is large and must have obtained as abundantly in the past. There have, on the other hand, been no important agencies which have served to eliminate it from the ocean. The great salt deposits, some of which are as old as the Cambrian, are,

* Forchhammer was the first to point out that potassium is being removed from the ocean, (British Association Report, 1844, p. 153.) From his analysis of Fucoids and of the metamorphosed Fucoid schists of Scandinavia he came to the conclusion that Fucoids constitute a very important factor in the process.

† Murray and Renaud, *op. cit.*, p. 389.

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as is certainly the case with the Stassfurt beds, the result of the evaporation of land-locked arms of the sea.* and they are constituted of but an infinitesimal fraction of what is contained in the ocean. Sodium chloride, like other constituents of sea water, is carried landward with evaporation and rain clouds, but it appears to be returned to the ocean, without any perceptible loss, through the river discharge. The only method of elimination which at all possibly counts is that in which it is imprisoned mechanically in the sedimentary deposits during their formation. That sodium chloride is removed in this way has been pointed out and emphasized by Osmond Fisher, but there are no data which serve to indicate that this is a considerable factor in diminishing the sodium content of the ocean. All the known facts point in the contrary direction. There is no mineral in the course of formation, which is extensive or abundant in its distribution and which also requires considerable quantities of sodium for its production, and there are, further, no agencies acting in the soils which serve to remove sodium compounds from the percolating water.

In these considerations we find a full explanation for the relative proportions (100 : 3.613) of the sodium and potassium which now obtain in sea water, and also for those which obtain in the river discharge of the globe. According to Murray's estimate for nineteen principal rivers, the proportions would be 100 : 38.6. We may postulate from this that in the early geological periods of the pre-Cambrian period, when soils did not exist, the quantities of each element discharged by rivers or bodies of water derived from the land surface, were nearly equal. Since the primeval ocean, as pointed out above, contained these elements in almost equal quantities, this condition must have continued until long after soils holding organisms and organic matter had appeared, and even for an indeterminable period after organisms had made the ocean their habitat. The change in the relative proportions once begun must have gone on with extreme slowness, and oceanic organisms, at first wholly of the unicellular kind, must have, after acquiring a relation to these elements, just as slowly responded to the changes in the proportions of their medium.

The river discharge of the globe has been from primeval times adding also magnesium and calcium to the sea. According to calculations based on Murray's data, the proportions relative to the sodium shown in these are 134 and 591 respectively to every 100 of the latter. This is, of course, based on approximate estimations, and they may be incorrect,

* See G. P. Merrill's "Treatise on Rock and Rock Weathering and Soils," p. 120, 1897.

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as they seem to be, if one scrutinizes the proportions that are found in rivers whose waters have been carefully analysed. There are only two rivers, the Amazon and the St. Lawrence, which give nearly the proportion of magnesium called for by Murray's estimates, while the Ottawa, the Mississippi, and the Nile give quantities much below that of the sodium, and the quantities of the calcium are found to vary very much for the different rivers. If we disregard Murray's estimates and base our observations on the analyses of the various rivers, we can safely conclude that, while the quantity of calcium added, except in the case of the Nile, is always, and sometimes very much, greater than the sodium addition, the latter does not probably exceed the amount of the magnesium discharged. In the ocean, however, the sodium, calcium, and magnesium have the proportions of 100, 3.91 and 12.0.

The comparatively low proportion of magnesium in sea water is explainable. In the first place, as pointed out above, there must have been in the primeval ocean but very little magnesium, owing to the conversion of all the chloride of magnesium into magnesia which is, except in minute quantities, insoluble. The conditions which so affected magnesium chloride left the chlorides of calcium, sodium and potassium unchanged, and in conseqence these went into solution in primeval sea water, and were, therefore, as compared with magnesium, very abundant. Further, the ocean at first must have contained only traces of the latter element and the subsequent addition of it through river discharge would increase the amount in sea water, but not to such an extent as to make it overtake the sodium.

There is another factor which operated in limiting the amount of the magnesium. This is the tendency shown by the chloride to interact with the carbonate of lime when the latter undergoes deposition to form limestone, and, in consequence, this always contains carbonate of magnesia. When the latter exceeds 10 per cent. the mixture of the carbonates is given the conventional name of dolomite, and in some formations of this kind the magnesia is found greatly to exceed the lime. Dolomites are found in all the periods down to and including the Cambrian and even in the pre-Cambrian, it is associated with the crystalline schists.* An exact estimation of the magnesium so localized is impossible, but on the average it cannot be more than 10 per cent. of the quantity of the calcium due to deposition, so that the amount of magnesium removed annually from sea water must fall far behind that of the calcium. It follows from this that whatever were

* Zirkel, Lehrbuch der Petrographie, 1894, Bd. 4, p. 499.

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the proportions of these two elements in primeval sea water, the proportions must have slowly changed, and as a consequence the magnesium must have gradually increased while the calcium practically remained stationary.

It must of course be admitted that magnesium is withdrawn from the ocean by organisms, but the amount thus removed is very small, and in no case is it an important method of eliminating the element from sea water. In the hard part of corals it is as a rule under one* per cent. and in the coralreefs it is less than that in amount, while the calcium constitutes nearly 40 per cent. Forchhammer's† analyses of the ash of sea weeds reveal a quantity of magnesium which he regarded as important, and he held that the Fucoids thus remove quantities of this element and deposit them in the beds which contain the solid substances of sea weeds as far as they are insoluble in water.‡ According to the analyses of Gödechens,§ the ash of Fucoids contains from 4 to 7 per cent. of magnesium. That the element is eliminated from sea water by these forms may be conceded, but it is doubtful if the quantity removed in this way is sufficient to affect materially in time the total amount retained in the ocean.

We may conclude, therefore, that in the formation of dolomites, of magnesia-holding limestones and chalk deposits, and, to a minor degree, in the activities of animals and plants, elimination of magnesium from sea water has always obtained; and, further, that the amount eliminated annually does not equal the amount of magnesium added to the sea by river discharge. This postulates a constant increase in the amount of magnesium in the sea; and in this respect it must be ranged with sodium, which increases in amount at a greater rate, since, so far as is known, there are for it no agencies of elimination in operation which compare with those affecting the potassium, the calcium, and even the magnesium. The sodium, therefore, though it is not added in greater amount than in the case of the latter, is increasing at a greater rate, and thus the proportion of sodium to magnesium in sea water is slowly altering. As pointed out above, the primeval ocean must have contained but an exceeding small quantity of magnesium, and the amount of the latter now in it is practically wholly derived from the leaching out of the land surfaces during the intervening ages.

As regards the calcium in sea water there is less uncertainty. The

* According to Forchhammer the corals, *Isis nobilis* and *Corallium nobile*, contain 6.36 and 2.1 per cent. respectively of magnesium carbonate.

† Roth, *op. cit.*, p. 616, where the results of analyses of a number of forms are given.

‡ *Op. cit.*, p. 159.

§ Ann. d Chem. und Pharm., Vol. 54, p. 351, 1854.

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calcium of river discharge greatly exceeds in amount that of the three other elements, and yet it is less abundant than either in the ocean. If there were no elimination of calcium from sea water, the salts of the latter element would long have reached the point of saturation in the ocean. The present condition is easy of explanation. On the one hand, calcium separates from sea water through the formation of sulphate and carbonate of lime, which are to a high degree insoluble. This constitutes in part the origin of the gypsum beds and of the limestones of sedimentary origin. On the other hand the myriads of organisms that have their habitat in the sea have the lime "habit," and they consequently remove from solution enormous quantities of calcium. This is the case not only with all forms provided with exoskeleta and endoskeleta, into the composition of which lime largely enters, but also with those which exercise the precipitating effect on the calcium salts they absorb from sea water, the precipitation rarely going so far as to form a distinct deposit in the cells or tissues of the organism. This power to precipitate is universal, as shown by the fact that the capacity to form calcareous skeleta is almost universal, and this capacity is merely an enhancement of the power to precipitate. The latter, therefore, operating so largely, separates calcium from sea water, and on the death and disintegration of the organisms, the element is deposited on the sea bottom either as phosphate or carbonate of calcium.* These deposits, owing to the fact that they contain few calciferous fossils, are regarded as due to chemical reactions alone; but if they are, sedimentary limestones should be of a more uniform distribution, whereas we find them more or less localized. The explanation that they are due to protoplasmic "secretion" and not to either chemical reaction or skeletal deposition in living forms accounts for much, and indicates what a factor living protoplasm, animal and vegetable, is in the separation of calcium from sea water.

Sterry Hunt† advanced the view that in the primeval ocean the chief salts were chlorides of calcium and magnesium, and that the constant, large output by river water of carbonates of sodium and potassium, and particularly of the former, affected a conversion of these into carbonate of lime and chlorides of sodium and potassium which were retained in solution, while the carbonate was deposited. The objection to this view is that, if it is correct, the conversion ought to have taken place in the pre-Cambrian period, and, therefore, there ought to be extensive limestone deposits in the rocks attributed to that period.

* Sterry Hunt, *op. cit.*, pp. 82 and 311.

† *Op. cit.*, pp. 2 and 41.

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There is, indeed, in these rocks only a small amount of crystalline or other limestone, and there appears to be still less in the divisions of the Huronian, which, as pointed out, have a thickness in the Lake of the Woods and Rainy Lake districts, according to Lawson,* aggregating 50,000 feet, all representing sedimentary formations. What limestone and gypsum are present in pre-Cambrian rocks can very well be attributed to the occurrence of calcium salts in the oceans of the Archæan in quantities, however, which could not have very greatly exceeded those which obtain to-day in sea water.

V.—THE RELATION OF THE SALTS IN THE OCEAN TO PROTOPLASM.

From the considerations advanced in the preceding section of this paper, it follows that the ocean has been, and is now, slowly changing, not in its composition, but in the proportions in it of the various elements to each other, and that, as a consequence, it is now in this respect greatly different from what the primeval ocean was in the period following the first condensations of water vapour on the rock crust of the globe. It may again be noted that in all these changes there are two distinct periods. In the first, or older, life was not represented except towards its close, and therefore, the only factors engaged in eliminating any of the elements from the sea were purely chemical ones such as are illustrated in the precipitation of lime as carbonate and sulphate and of magnesia as carbonate. In this period the elements must have differed in amounts from each other less markedly than they do to-day, and the constant addition to these from the discharge from the land surfaces did not tend to alter, even after a very long interval, the proportions which first obtained. This period must have terminated some considerable time after the appearance of living forms on the globe, and especially only after the adaptation of vegetable forms to a land life, and the consequent production of soils. The second period could not have begun at once after the appearance of living forms, for these must first have acquired a relation to the elements and then have developed the habit of disposing of the various salts which they took out of the sea water. This period may well be supposed to have begun when there had developed not only a considerable diversity of forms in the sea, but also the organisms which contribute to the production of organic matter in soils. In this period the removal of potassium from the land surfaces decreased,

* *Loc cit.*

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and the combination of this element with argillaceous matter at the bottom of shallow portions of the sea began. As a result the amount of potassium in sea water became stationary. At the same time the removal of calcium on a larger scale than obtained in the preceding period commenced and this checked the increase of calcium salts.

The first forms of life in the primeval ocean were undoubtedly unicellular, and they were probably also organisms which presented features intermediate between those of the vegetable kingdom on the one hand, and those of the animal kingdom on the other. These forms must have persisted for a period of unknown but very great duration, for in them developed not only a nucleus but also the capacity on the part of the latter to divide in the remarkable and complicated manner illustrative of karyokinesis, and which is characteristic now of the cells of both kingdoms. This process of division, so alike in its main features in animal and vegetable cells, must have become fixed before specialization had gone so far as to evolve both animal and vegetable types, for, had it been otherwise, there would have been greater differences in the process in animal and vegetable forms. That the process has continued practically unchanged in all the intervening millions of years shows how deeply fixed in the organism this morphological habit has become, and, therefore, the act of fixation must have taken an incredibly long period of time during which the ocean was changing, not in the relative proportions to each of the elements it contained, but in the absolute amounts of these.

During this long period, these organisms, neither distinctly animal nor distinctly vegetable, exposed as they were to action of these same elements, must have acquired a relation to them as fixed as the karyokinetic process was becoming. Their protoplasm had established all its normal processes in the presence of potassium, sodium, calcium, and magnesium in certain proportions in sea water, and, after the lapse of the long period of time required for the elaboration of the karyokinetic method of division, these processes became unalterably dependent on the presence of the elements in the proportions which then prevailed. Without this fixed relation life could not continue, and when specialization into animal and vegetable forms occurred this fixed relation was transmitted to the forms of both kingdoms. How long these latter forms remained unicellular cannot, of course, be surmised, for there are no means of determining the length in time of this or any part of the pre-Cambrian age, but that it was of very great duration can hardly be questioned, and it must have strengthened the relation which obtained between protoplasm on the one hand and the elements in certain pro-

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portions, on the other.* In consequence, their descendant forms inherited this relation, and transmitted it to the forms and species which arose through variation and other causes. When multicellular forms arose these were endowed with the same relation.

The proportions of the elements in the early pre-Cambrian ocean with their long action on protoplasm must then have conferred a more or less fixed property on the latter and, in consequence, living matter, whether animal or vegetable, now shows in its ash proportions of the elements greatly different from those found in the media in which it lives or in the circulatory fluid which bathes it. This relation or property resists change even after exposure to altered conditions for a very long period of time. Before the circulatory fluid (blood plasma) was established in multicellular animals, a great change must have occurred in the proportions of the elements in the ocean, a change which would account for the wide differences between the proportions in the protoplasm or tissue on the one hand, and those in the blood on the other.

The proportions of the elements in living matter are due then to conditions which obtained in the ocean far back in the pre-Cambrian age, while those in the blood or plasma are due to conditions which occurred in the ocean long after this and yet before the beginning of the Cambrian period. The proportion of potassium to sodium in blood plasma is nearly† double what it is in the ocean and therefore that difference must have resulted in the period that has elapsed since the rudiments of a circulatory system were developed in those Metazoan animals which gave rise to Vertebrates.

As pointed out above, it is difficult to obtain the exact proportions of the sodium, potassium, calcium and magnesium in living matter, for, except in muscle fibre, protoplasmic structures cannot in sufficient quantities be freed from adherent material which carries these elements in very different proportions. Calcium exists in tissues apart from the protoplasm and as precipitates or deposits, and according to recent observations which I have made, this is true in a very large degree of

* Geologists concede a very long time to the pre-Cambrian, a duration which, according to the different estimates, ranges from one-third to four-fifths, and even nine-tenths, of the whole geological period. The very fact that all the chief types of animal life, and perhaps also of vegetable life as well, appeared before the close of the pre-Cambrian age, indicated that the latter was of inconceivably long duration.

† Amongst the oldest and highly specialized forms are *Olenellus* and the Brachiopods of the Cambrian. The oldest Vertebrate remains are in the Trenton division of the Silurian, more recent than the Cambrian, but these are "ganoid" in character and this fact postulates a long preceding period of development out of Protovertebrate forms which therefore could not have first appeared much later than the beginning of the Cambrian. The circulatory system of Vertebrates accordingly has a history which began in the pre-Cambrian age.

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potassium. With regard to this element it may be said that active living matter has the power of absorbing it in large quantities and disposing of it in an inert form by precipitating it at the peripheries of cells or in inert organic masses within them, and, as a consequence, the ash of animal and vegetable cells shows a larger quantity of potassium than the protoplasm of the cells required. This illustrates how difficult it is to determine the primitive and fixed proportions of potassium and calcium, and further, how little we should depend, even in the case of muscle, on the analyses of the ash of organs or organisms, for this purpose. If a sufficient quantity of Amœbæ* could be obtained for analysis it might yield results of value but until that is done, the exact proportions of sodium, potassium, calcium and magnesium must be a matter for conjecture. It can scarcely be that the proportions found in muscle represent even approximately those which should obtain in undifferentiated propoplasm or cells.†

VI.—EVIDENCE FROM THE LAKES AND RIVERS OF THE PRESENT PERIOD.

It may be pointed out that in the composition of the rivers, large lakes, and seas of the world, there is evidence confirmatory of the view that potassium and calcium predominated in the pre-Cambrian seas. The conditions, of course, which contribute to the composition of the lakes of to-day are not the same as those which existed when the oceans of the globe were formed. There are but infinitesimal traces of the chlorides of calcium and potassium in the rock crust or sedimentary strata, and, further, there are, apart from the deposits of salt, and that amount of it due to rainfall, but small quantities of sodium chloride which can to-day come under the leaching action of water. There are also soils to alter the proportions of the chemical elements derived from them.

Nevertheless it happens that in lakes surrounded, either wholly or

* Fresh water, unicellular animal organisms are apparently free from excess of the elements. They are, as a rule, free from potassium, at least in such quantities as are found in other organisms.

† According to J. Katz (Pfluger's Arch., vol. 63, p. 1), the proportions in muscle from different animals are:

	Na.	*K.*	*Ca.*	*Mg.*
Man	100	400	9.3	26.4
Dog	100	354	7.26	25.1
Rabbit	100	870	40.0	60.5
Pike	100	1415	145.0	105.0

These and other results of the same observer are open to the objection that no effort was made to get muscle fibre free from all adherent tissues. Visible blood vessels, tendons, nerves and fat were indeed removed but these constitute only a part of the non-muscle portions of the tissue and they may be the cause of the variations shown in the results.

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partially, by regions in which pre-Cambrian formations occur, or are the only apparent rocks, the potassium predominates over the sodium. For example, in the water from Reindeer Lake, which is situated 400 miles directly north of Lake Winnipeg, Professor Adams found the potassium to exceed very greatly the sodium. In the water from the Churchill River, as well as in the water from the Saskatchewan River above the junction of the Big Stone River, the potassium is much richer than the sodium.* These rivers drain rocky areas chiefly of the pre-Cambrian type. Rocks of the primitive kind, therefore, contrary to the prevailing opinion,† supply to the water which comes in contact with them more potassium than sodium.

Even in the case of Lake Superior which draws its supply not only from the primitive rock region on its northern side, but also from the areas covered with soils of alluvial and drift origin on the south, the potassium is about equivalent to the sodium. In the lakes of the Bavarian Highlands, Rachel See, Würm See and Ronig See, the potassium is twice in amount that of the sodium. In Lake Zurich the potassium exceeds the sodium. In Lake Geneva, in Pyrenean and Vosgean Lakes and in those of Russia, Armenia and Central Asia the potassium is approximately two-thirds of the sodium. It is probable that if proper methods for estimating potassium had been current in his day, C. Schmidt would have found for the lakes of Russia, Armenia and Central Asia a higher potassium value than he obtained, for the methods then in vogue for the determination of the element in the presence of sodium were very faulty and gave very low results. It is probable also that this may explain the low value found by Sterry Hunt for the potassium of the Ottawa River, whose waters, as well known, are derived largely from Archæan regions.

The Tables A and B show further that, in nearly all cases, the calcium is very abundant. In the Nile only, amongst the rivers, is it less than the sodium, while it very greatly exceeds it in the rest. In the lakes it is very abundant relatively, with the exception of the Rachel See and Lake Onega. In the Bavarian lakes, Lake Geneva, Lake Zurich, and some others, it is exceedingly abundant relatively.

The magnesium is always less than the calcium, and the relative difference is sometimes very great. It may fall below the sodium, but, as a rule, it is greater in amount.

These proportions, one can readily understand, must have been

* F. D. Adams Geo. and Nat. Hist. Survey of Canada, 1880-2, p. 6, 4.

† This opinion is based largely on the fact that the potash feldspars are difficult to decompose while the soda feldspars readily undergo decomposition.

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uniformly maintained for an indefinitely long period. It may even be claimed that, in the case of Lake Baikal, of Reindeer Lake, and other lakes supplied from Archæan areas the proportions have obtained from pre-Cambrian times, and further, that the river discharge of that period, coming as it did from pre-Cambrian rock areas wholly, would contain the four elements in these or similar proportions. That would postulate that the primeval ocean was merely a gigantic body of fresh water, in which the sodium, potassium, calcium, and magnesium obtained in quantities and proportions as they now obtain in a lake situated in Archæan area. As already pointed out, these proportions gave place to others, and to-day, as in the past, the relative amounts of each element are changing, so that in a few million years hence the composition of ocean water will be appreciably different from what it is now.

One can, indeed, illustrate what changes have taken place in the ocean by reference to such a large body of fresh water as Lake Superior. If the latter were to lose its outlet no doubt its area would be larger than it is now, but when that had attained a certain extent the evaporation would balance the inflow as in the case of the Caspian Sea, and in consequence the salts held in solution would constantly increase in amount, but each at different rates up to a certain point, when the proportions would begin to approximate those in ocean water. One cannot of course say that this is what has happened in the case of either the Caspian or the Sea of Aral, for these bodies of water were connected with the ocean as late as the beginning of the Tertiary age, but it may be pointed out that if their composition was, to start with, the same as that of the ocean in Tertiary times, their present composition is strong evidence of the effect that the salts derived from leaching of the land areas have in modifying the proportions, for in that respect either is markedly different from the other and from the ocean.

The Great Salt Lake of Utah may be adduced as an instance of the change of a body of fresh water into one which presents a high degree of salinity and which in the proportions of its salts is remarkably not unlike the ocean. This lake, which is in part of the area covered by the glacial Lake Bonneville, is considered by G. K. Gilbert to have been a body of fresh water about 25,000 years ago. He arrived at this result by determining the discharge of chlorine into the lake by river water and comparing it with the quantity at present obtaining in the lake. Lake Bonneville had* an outlet delivering its waters into a tributary of the Columbia River and thus the lake was kept fresh. When, however, this outlet was lost, changes climatic and physical operated to reduce

* Monographs of the U. S. Geol. Survey, Vol. 1, Lake Bonneville, 1890, p. 254.

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the volume of water, and, evaporation keeping pace with the inflow, a concentration of the salts held in solution took place. An examination of the present sources of inflow shows that these do not contain the sodium, potassium, calcium and magnesium in the relative proportions which are found in the lake. Gilbert estimates that it would take only eighteen years to give the lake through its fresh water inflow, all the calcium it now contains and that 850 years would to this end be required for magnesium. He does not deal with the case of the potassium of which the analyses he reports show only traces in the inflow water, but this also may have been due to faulty methods of determining that element. These latter seem to be the only explanation for the great discrepancy between the amounts of potassium found by Talmage* in 1889 and Bassett† in 1873‡.

Short as is the extreme period required by Gilbert's calculations to affect all the changes in the composition, it has epitomized the history of the ocean. Even if we postulate that the primitive rock crust of the globe in pre-Cambrian times contained more sodium chloride than what is found now in Archæan formations, there is also more of this salt in the strata of later geological periods which cover the drainage area of Utah Salt Lake. Of course there is not a complete parallel between the latter and the ocean, for the relative proportions are not exactly the same, but their approximate similarity is striking, and, it may be added, very convincing as to the extreme probability of the thesis maintained above.§

TABLE A.

RIVERS.

	Na.	*K.*	*Ca.*	*Mg.*	*SO3.*	*Cl.*	*Si.*	*Fe.*
1. St. Lawrence ...	100	22.9	638.0	143.4	136.0	223.0	343.0	....
2. Ottawa	100	64.2	416.7	82.5	67.3	224.3	402.0	....
3. Mississippi	100	35.5	462.0	82.0	17.1	8.4	86.4	17.0
4. Amazons.....	100	72.6	1,089.0	135.6	36.0	90.0	....	....
5. Nile	100	22.2	75.1	41.5	18.5	16.0	44.4	....
6. Assinaboine.....	100	10.5	122.0	69.4	127.9	50.0	....	....
7. Red River	100	12.3	133.3	83.2	190.0	91.4	....	....
8. Nineteen Rivers (Murray)	100	38.6	590.9	134.2	197.6	53.5	145.3	37.9

* Science, Vol. 14, 1889, p. 445.

† Chemical News, Vol. 28, 1873, p. 236.

‡ See Table B, Utah Salt Lake, 26 and 27.

§ In Lake Shirwa, according to J. E. S. Moore, ("The Tanganyika Problem," 1903, p. 22,) we have a lake which was once fresh, but has become salt through the loss of its outlet. So far as I know no analyses have been made of its waters.

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TABLE B.

LAKES AND SEAS.

	Na.	*K.*	*Ca.*	*Mg.*	*SO3.*	*Cl.*	*Si.*	*Fe.*
9. Superior	100	97.2	1,015.0	208.0	27.5	103.2	19.3	....
10. Rachel See	100	199.4	13.0	....	....	17.5	....	1.6
11. Würm See	100	223.9	2,445.0	809.0	....	....	....	....
12. Walchen See....	100	117.7	2,557.0	808.5	....	....	....	....
13. Ronig See	100	207.9	5,812.0	588.8	....	....	....	....
14. Schlier See.....	100	96.4	3,141.0	661.0	....	....	....	....
15. Lac Gaube (Pyrenean).....	100	66.3	421.0	17.9	....	....	....	....
16. Lac Gerardmer (Vosges).....	100	70.4	117.6	29.8	....	....	....	....
17. Lake Geneva....	100	74.5	2,345.0	341.3	....	55.0	1,806.0	....
18. Lake Zurich.....	100	111.7	1,843.9	272.0	441.0	36.0	60.8	....
19. Lake Peipus.....	100	75.2	929.5	159.9	18.5	134.0	13.3	3.4
20. Lake Onega	100	71.4	67.3	51.4	33.4	104.7	36.9	2.2
21. Lake Tschaldyr (Armenian Highlands)	100	59.3	219.3	45.7	88.2	65.3	172.1	5.5
22. Lake Baikal.....	100	58.9	399.6	60.6	98.5	41.7	16.1	17.3
23. Sea of Aral	100	2.38	18.6	24.2	113.0	156.0	....	....
24. Caspian Sea.....	100	3.35	9.4	22.7	80.0	163.7	....	....
25. Dead Sea	100	28.4	40.3	1.27	2.0	556.1	....	....
26. Utah Salt Lake..	100	3.22	1.22	7.81	14.9	169.2	....	....
27. " " ..	100	25.8	1.56	7.82	19.1	192.2	....	....
28. Ocean	100	3.613	3.91	12.0	20.9	180.9	....	....

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SUMMARY.

The points discussed in the preceding pages may be summarized as follows :—

1. The composition of the ocean represents the result, on the one hand, of the leaching action of water on the land surfaces of the globe continued throughout all the geological periods, and, on the other, of the chemical and other agencies modifying or enhancing the power of sea water to retain in solution the mineral constituents derived from the land surfaces through river water since the beginning of the primeval period.

2. The relative proportions of the elements, and especially of sodium, potassium, calcium, and magnesium, in river discharge are not parallel to those of the same elements found in the sea. In river water, the calcium is always more, and the potassium less, abundant than the sodium, while the magnesium appears to approximate in amount the latter. In the sea, on the other hand, the sodium is much more abundant than the other three elements, and this is due to the continuous precipitation of a very great portion of the calcium added by rivers as carbonate, to the subsequent fixation in the limestone so formed of the magnesium as carbonate, and to the removal, continually taking place, of potassium, which is affected through animal and vegetable forms, and its consequent fixation in submarine deposits as glauconite and other potassium-holding minerals. The calcium and potassium appear to be stationary in amount, while the magnesium added by river water appears to exceed in amount that removed from the sea, and, in consequence, is slowly on the increase in the ocean, but its rate of increase is far behind that of the sodium.

3. The relative proportions of the elements in the ocean have, therefore, always been changing, and these proportions must have been, in the earlier geological periods, very different from what they are now. In the ocean of the earliest period the relative proportions of the elements approximated those found in river discharge, or rather those found in fresh water shed from areas covered with Archæan rocks. In this the potassium approaches the sodium in amount while the magnesium exceeds the latter, and the calcium is relatively very abundant.

4. This condition must have continued until living forms made

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their appearance in the ocean when the gradual elimination of the magnesium, and particularly of the potassium and calcium, began. The forms were in all probability unicellular, and as the period must have been of great duration, the organisms and their protoplasm acquired a fixed relation to the four elements.

5. With the appearance of vegetable land forms and the formation of soils the removal of potassium from the land to the sea by river water diminished, and this, in conjunction with the elimination of the element from sea water by organisms, made the amount in the sea stationary. Through the action of living forms the calcium also in sea water has been kept stationary since that remote period.

6. In the transition from the ocean of the more ancient composition to that of the present, the unicellular forms became multicellular, and developed circulatory systems, the vascular fluids of which were at first simply modified sea water. In the blood plasma of Vertebrates, the three elements, sodium, potassium, and calcium are in relative proportions strikingly like those which now obtain in sea water. The magnesium only is considerably less than it is in sea water. The whole is due to heredity, the proportions of the saline constituents of the plasma being a reproduction of the proportions which obtained in sea water when circulatory plasmata were developed.

7. The proportions of the four elements which obtain in living protoplasm are as yet unknown, for the latter has the power of precipitating the potassium, calcium and probably the sodium and magnesium as inert compounds in itself or in its adventitious structures, and thus analyses would comprehend the inert material as well as the quantities of these elements which are actively participating in the processes of the living substance. If we could determine the latter quantities alone we could regard them as a representation of the proportions obtaining in primeval sea water to which the protoplasm of unicellular organisms had established a fixed relation.

8. That such a relation could be inherited may be inferred from the fact that the karyokinetic process, being practically the same in the animal and vegetable cell, has continued unchanged in both from the primeval period when the karyokinetic process first developed in a parent unicellular organism neither distinctly animal nor distinctly vegetable. This indicates how marked an influence heredity wields.

9. Briefly, animal as well as vegetable protoplasm owes its relations

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to the elements sodium, potassium, calcium and magnesium to the composition of sea water which obtained when all forms were unicellular just as the blood plasma owes its relations to the same four elements to the composition of sea water which prevailed when circulatory fluids were established. In other words the relation of protoplasm to salts is due to the action for ages of sea water, for incalculably long periods of time, on the living matter of unicellular organisms.

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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. E. L. MARK, DIRECTOR.

No. 139.

A PARAFFINE BATH HEATED BY ELECTRICITY.

BY E. L. MARK.

WITH THREE FIGURES.

FROM THE AMERICAN NATURALIST, VOL. XXXVII. No. 434.

CAMBRIDGE, MASS., U. S. A.
FEBRUARY, 1903.


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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE, E. L. MARK, DIRECTOR.— No. 139.

A PARAFFINE BATH HEATED BY ELECTRICITY.

E. L. MARK.

UNDER the stimulus of disastrous explosions of gas in other museums and some unpleasant experiences in our own, it was decided somewhat over a year ago to replace, as far as practicable, heating by gas in the Museum laboratories with heating by electricity. The greatest danger from the use of gas is incurred where two or more lights are kept constantly burning in the same room. All of our constant burners for heating water-baths, warm ovens, etc., have been for many years supplied with the Koch automatic cut off, so that in case of accidental extinguishing of the flame, the cooling down of the burner would automatically shut off the gas supply. But the lever of the Koch burner will not always work, even though loaded with a weight greater than that which it carries when it comes from the maker; moreover, the metal, on the expansion and contraction of which the tripping apparatus depends, after a time loses to a certain extent its expansive properties, so that it fails to release the lever and cut off the gas.

The Departments of Botany and of Zoölogy were already in possession of a number of copper water-baths for heating paraffine and warm ovens of various constructions, which it was desirable to retain if they could be provided with a suitable electric heating apparatus. With the aid of suggestions from Professor Sabine of the Department of Physics and the coöperation of constructors of electrical apparatus, a plan was finally worked out which answers fairly well the requirements of the ordinary paraffine water-baths.

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There were two chief problems to be solved: first, to ascertain the minimum heating capacity of the heating coil necessary for each bath; secondly, to devise an automatic regulator to control the electric heating current. To reduce the amount of heat lost, each bath was provided with a felt jacket,— covering all parts except the top,— enclosed in a canvas cover. The felt was about half an inch thick, and the canvas cover after

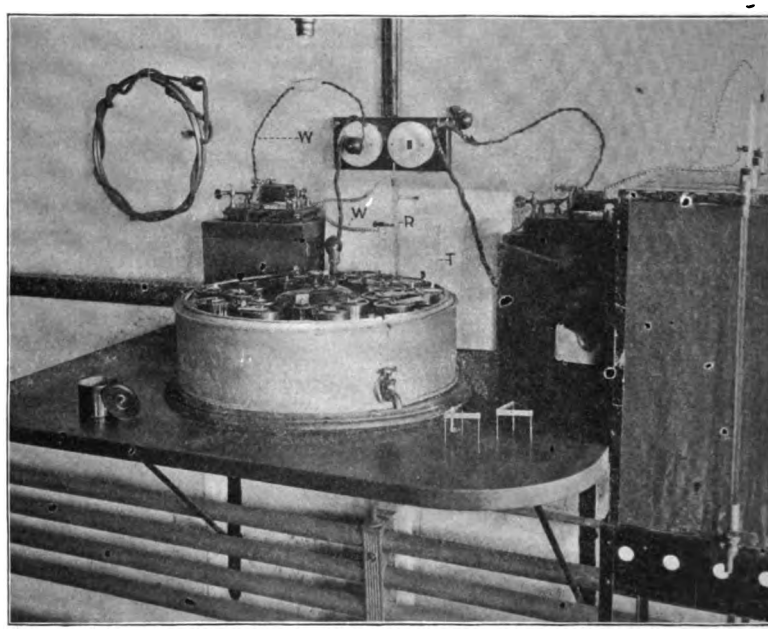


FIG. 1. — Paraffine water-bath and automatic electric heating apparatus. The 150-ohm telegraphic relay apparatus is mounted on the top of box containing a one-cell battery. Plug in place in the receptacle. At the right the plug of another heating apparatus removed from receptacle and hung on a hook. At the left a detached heating coil and insulated wire cable hanging on the wall. *R.*, regulator; *T.*, thermometer; *W.*, wires of heating circuit; *W'.*, wires of relay circuit.

being slipped over the felt was laced up on one side, as is shown above the faucet in Figure 1. The manufacturers of the heating apparatus employed (the Simplex Electric Co. of Boston and Cambridge) then determined empirically the proper resistance and length of coil required to maintain a given temperature in each of the different forms and sizes of baths. To insure uniform distribution of heat, the coil should make at least one whole

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turn. As constructed for our paraffine baths the coil is a somewhat flexible cylinder, about half an inch in diameter, with copper covering. With its attached insulated wires such a coil is shown hanging on the wall at the left in Figure 1. The resistance metal used in the coil was a copper-nickel alloy. In the case of the paraffine baths this coil was simply shoved into the bath through an inch hole made in the top of the bath. The knob with connecting wires protrudes outside, and the coil rests on the floor of the bath, immersed, of course, in water. In the case of the thermostats used for incubating purposes, etc., a hole was cut in the side or bottom of the water reservoir, and the coil after being introduced was soldered in place so as to close the hole.

To devise a regulator was more difficult. The use of an ordinary rheostat proved to be impracticable, because it was not possible to make sufficiently fine gradations of resistance for different temperatures, nor by it to provide against fluctuations in the initial current.

The method finally adopted utilizes a one-cell relay battery to magnetize an electro-magnet. The heating current is made to pass through the armature, which is pulled into contact with a vertical post by means of a delicate spring. When the armature is drawn away from the post by the magnet the heating current is broken. Into the circuit of the relay battery is put the regulator; when the relay circuit is closed at the regulator, the magnet operates on the armature and breaks the heating current; when the relay circuit is broken at the regulator, the armature is drawn back by the spring to its first position thus closing the heating circuit.

Our Reichert gas regulators (*R*), already in use for the purpose of regulating the *gas* supply to the burners, were adapted by very slight changes to the new requirements. The tubular glass stopper carrying the gas inlet was removed; in its place was put a cork, bored to receive one of the copper wires (*W'*) from the single-cell battery of the Leclanche type (Samson cell No. 2). The copper wire terminates with a No. 20 platinum wire (Fig. 3, *Pt.*) which is about an inch long; the height of the lower end of this wire above the mercury in the column can be

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roughly adjusted by moving the copper wire through the cork and wedging it in place by a small wooden wedge occupying with the wire the hole in the cork. The contact of the mercury with the platinum wire serves to close the circuit of the battery, the other wire from the battery being connected to the mercury by means of the screw and piston in the side tube used to adjust the height of the mercury column. Into this single-cell circuit is put a standard 150-ohm telegraphic relay machine of the pattern used by the Western Union Telegraph Co.

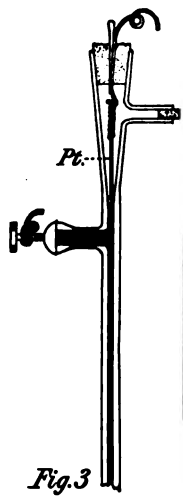


Fig. 3

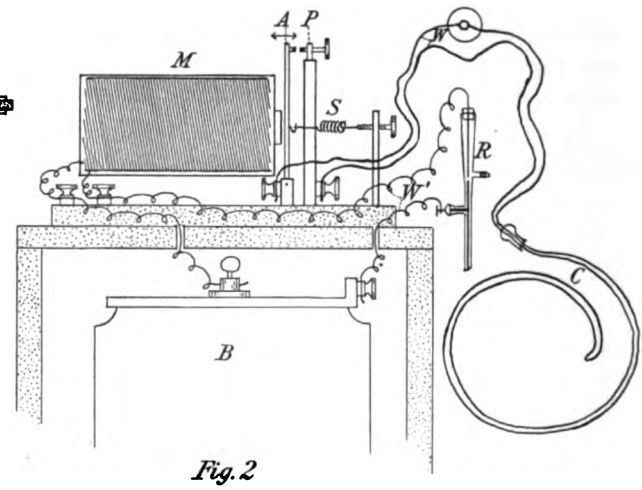


Fig. 2

FIG. 2. — Diagram to show the connections of heating coil and circuit with relay battery, electro-magnet and mercurial regulator. *A.*, armature; *B.*, battery; *C.*, heating coil; *M.*, electro-magnet; *P.*, post in the heating circuit; *R.*, regulator; *S.*, spring; *W.*, wire of the heating circuit; *W'.*, wire of the relay circuit.

FIG. 3. — Upper part of mercurial regulator — Reichert's gas regulator modified — drawn to larger scale than in Figure 2. *Pt.*, platinum wire.

The closure of this single-cell circuit magnetizes the core of the electro-magnet (*M.*), which, pulling against the delicate spring (*S.*), overcomes it and moves the armature (*A.*) away from the post (*P.*), thereby *breaking* the heating current.

When, owing to the interruption of the heating current, the bath cools, the mercury in the regulator recedes from the platinum point, thus breaking the *relay* circuit, the electro-magnet becomes demagnetized, and the delicate spring pulls the armature into contact with the post and thus closes the *heating* current. The finer adjustment of the distance between platinum point and

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mercury in the regulator is effected, as in gas regulation, by the screw and piston working on the mercury in the horizontal arm of the regulator.

The points on armature and post at which the heating current (110 volts alternating) is made or broken require to be made of heavy platinum wire (No. 15), for otherwise there is danger that the metal will fuse.

The paraffine baths of the form shown in Figure 1 are about 19 inches in diameter and 5 inches deep. The current required to heat such a bath is approximately equal to that of four 16-candle-power lamps. This could be much reduced by enclosing the bath in a glazed frame, one side of which would have to be movable to permit access to the cups.

The whole apparatus, except the heating coil, was furnished and installed by Clark & Mills, 23 Church St., Cambridge, and 543 Boylston St., Boston. It cost, including the heating coils, between $25.00 and $30.00 for each bath.

In determining the resistance to be used in the heating coil, one should make it as low as possible consistent with the maximum heating capacity required; for with greater heating capacity the heating current will be in operation a shorter time than with less heating capacity, and consequently the *relay* current — which is in operation whenever the heating current is not — will be required to work longer, and therefore the battery will become exhausted sooner. When the battery is so far exhausted that it will no longer magnetize the core sufficiently to overcome the spring and break the current, the regulation fails and too high a temperature results. However, a single cell will, with proper care, last for several months without renewal. To guard against the danger of too weak a relay current, one should test the current from time to time with a voltmeter.

There is one difficulty with this mercurial regulator, due to the oxidation of the mercury at the time of making and breaking the relay current. This in time causes a deposit on the platinum wire of oxide, which acts as an insulator and thus prevents sharp contact. The deposit may be removed, however, by immersing the platinum wire from time to time in weak nitric acid, and if a layer of high-test kerosene oil covers the mercury in the regulator the oxidation may be prevented.


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E. L. MARK, *Director*.

*** Abbreviations used:—

B. M. C. Z. . . . . for Bull. Mus. Comp. Zoöl.
P. A. A. . . . . for Proceed. Amer. Acad. Arts and Sci.
P. B. S. N. H. . . . . for Proceed. Bost. Soc. Nat. Hist.

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Über die Bedeutung der Blutkörperchen für die Blutgerinnung und die Entzündung einiger Arthropoden und über mechanische Einwirkungen auf das Protoplasma dieser Zellen.

(Aus dem Marine Biological Laboratory, Woods Holl, Mass, und dem pathologischen Laboratorium der McGill University, Montreal Canada.)

Von

Leo Loeb.

Im folgenden sollen einige Beobachtungen und Versuche über die Blutgerinnung und das Verhalten der Blutkörperchen bei der Entzündung einiger Arthropoden mitgeteilt werden.[1]) Diese Versuche wurden an Limulus polyphemus, Homarus americanus, Platyonychus ocellatus und einigen anderen Tieren ausgeführt. Bei diesen Tieren liegen drei verschiedene Typen der Blutcoagulation vor. Zuerst soll die makroskopische und mikroskopische Erscheinungsweise der normalen Koagulation, sodann

[1]) Die Versuche wurden Ende Sommer 1901 im Biologischen Laboratorium zu Woods Holl, Mass. begonnen und diesen Sommer (1902) dort weitergeführt und im pathologischen Laboratorium der McGill University abgeschlossen.



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der durch verschiedene Agentien beeinflußten Gerinnung beschrieben werden. Darauf werden Versuche über Beeinflussung einer bestimmten Phase der Koagulation des Hummerblutes mitgeteilt. Weiterhin wird über das Verhalten der Blutkörperchen gegenüber in Limulus eingeführten Fremdkörpern berichtet, und zuletzt auf gewisse Analogien hingewiesen, die zwischen den hier beschriebenen Vorgängen und normalen und pathologischen Zell- und Gewebsvorgängen bei Wirbeltieren bestehen. Kurze Mitteilungen über die Blutkoagulation von verschiedenen wirbellosen Tieren liegen vor von L. Frédéricq, P. Geddes, der die Syncytiumbildung beschrieb, E. A. Schäfer, der auf das Vorkommen einer der Säugetierblutkoagulation ähnlichen Gerinnung neben der Syncytiumbildung hinwies, ferner von Pouchet, sowie von Haycraft und Carlier. Ausführlichere Mitteilungen liegen vor von Halliburton, M. Loewit und W. B. Hardy. In einer früheren Arbeit beschrieb ich[1]) die an den Blutzellen nach dem Verlassen des Körpers eintretenden Veränderungen, insbesondere die Beziehungen zwischen den Veränderungen der Granula und der übrigen Zellbestandteile, ferner den Einfluß verschiedener Substanzen, wie Säuren und Alkalien auf die Blutzellen, sowie das Verhalten der letzteren gegen in den Körper eingeführte Fremdkörper.

Nachdem diese Arbeit der Hauptsache nach abgeschlossen war, erhielt ich Kenntnis von einem Autoreferat einer Arbeit P. Bottazzis[2]) über Blutgerinnung bei Crustaceen, sowie einer Mitteilung Gautiers[3]) über den Charakter mechanisch hervorgerufener Eiweißgerinnsel.

Der normale Verlauf der Gerinnung des Blutes.

Sobald das Blut bei gewöhnlicher Temperatur den Tierkörper verläßt, setzen innerhalb weniger Sekunden Veränderungen ein, die zur Gerinnung führen. Viele Zellen werden schon verändert, während das Blut herausfließt. Die Geschwindigkeit des herausströmenden Blutes ist am größten in einem erwachsenen

[1]) On the blood lymphcells and inflammatory processes of limulus. The Journal of Med. Resarch. Vol. II. 1902. (Siehe hier Literatur.)

[2]) Centralblatt f. Physiologie 1902. No. 13.

[3]) Comptes rendus de l'Académie des Sciences 1903. Vol. 135. No. 3.

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alten Limulus heraus, jedenfalls kann hier die Menge des centralen, nicht mit dem Gewebe und der Außenfläche des Tieres in Berührung kommenden Blutes nur klein sein; ebenso langsam in dem Hummer, und oft nur tropfenweise in Libinia. Am leichtesten ist daher das Blut, insbesondere das Verhalten der Blutzellen und der Gerinnung experimentell am erwachsenen Limulus zu beeinflussen. Bei den anderen Tieren geht das Blut schon Veränderungen ein, bevor es der experimentellen Beeinflussung zugänglich ist. Fängt man nun das Blut in Schalen auf, so sieht man, falls Limulus benutzt wurde, das Blut im Laufe der ersten Minuten zu einer gelatinösen Masse werden. Der Vorgang beginnt, sobald das Blut den Körper verläßt. Dann retrahiert sich allmählich im Laufe der nächsten Stunden das gebildete gelatinöse Coagulum mehr und mehr und preßt das Serum aus; und am nächsten Tage hat sich gewöhnlich das voluminöse Coagulum auf einen ganz kleinen, runden Fibrinkörper retrahiert, welcher in seinem physikalischen Verhalten ganz dem Faserstoff der Säugetiere gleicht.

Beim Erwärmen mit Alkalien quillt das Fibrin auf, mit Säuren hingegen nicht. Bei stärkerem Erhitzen wird es weiß und undurchsichtig, also findet wohl eine Gerinnung statt. Eine Kupferoxyd reduzierende Gruppe konnte durch Kochen mit 5 pCt. Kaliumhydroxyd während einer Stunde, in während dieser Zeit wiederholt entnommenen Proben nicht nachgewiesen werden. Also chemisch ist diese im Anfang schleimig erscheinende Masse nicht ein Mucin.[1]) Häufig nun findet man, nachdem dieses erste Coagulum sich schon großenteils retrahiert hat, ringsherum lockere gallertige Massen dem centralen Pfropf sich anschließen. Meist sind diese sekundären Gerinnsel nicht sehr bedeutend, zuweilen aber sind sie doch so massig, daß das ganze ausgedrückte Serum wiederum von diesem zweiten Coagulum eingeschlossen wird, sodaß dann außer der ersten Koagulation nach einigen Stunden noch einmal eine vollständige zweite Koagulation des ausge-

[1]) Einige dieser Versuche führte ich in dem unter der Leitung von Herrn Dr. O. Folin stehenden physiologisch-chemischen Laboratorium des McLean Hospital in Waverley aus. Herrn Dr. Folin bin ich für seine Freundlichkeit zu großem Dank verpflichtet.



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schiedenen Serums stattfinden kann. Während also bei Limulus diese Nachgerinnung gewöhnlich sehr unbedeutend ist, und gleich im Beginn die Bildung einer massigen Gallerte stattfindet, ist beim Hummer die erste Gerinnung geringfügig. Gewöhnlich bildet sich in einer größeren Menge Flüssigkeit ein Fibrinnetz, das sich herausfischen läßt. Hier findet gewöhnlich keine gallertige Gerinnung direkt nach dem Ausfließen des Blutes statt. Zuweilen wird aber auch hier gleich im Anfang die Gerinnung massiger. Es findet aber beim Hummer in sehr deutlicher Weise eine Nachgerinnung statt, die, wie wir sahen, bei Limulus gewöhnlich eine sehr unbedeutende Rolle spielt. Gewöhnlich kann man die Fäden der ersten Gerinnung durch Schütteln der Schale, in der das Blut aufgefangen wird, leicht sammeln. Dieses erste Gerinnsel ist beim Hummer an Masse gewöhnlich im Vergleich zu der zurückbleibenden Flüssigkeit unbedeutend. Dann aber tritt hier im Gegensatz zum Limulus eine sehr ausgesprochene Nachgerinnung ein. Nach verschieden langer Zeit, die von etwa 10 Minuten bis zu Stunden variieren kann, gerinnt diese restierende Flüssigkeit zu einer gelatinösen Masse, die sich nur langsam retrahiert. In seltenen Fällen kann diese Nachgerinnung ganz ausbleiben. Bei den Krebsen (Libinia, Platyonychus) gleicht die erste Gerinnung der ersten Koagulation des Hummers, eine zweite Gerinnung hingegen sah ich hier nie eintreten, die restierende Flüssigkeit veränderte ihren Zustand nicht weiter.

Die mikroskopischen Veränderungen während der Blutgerinnung.

Verfolgen wir das Verhalten der Blutkörperchen unter dem Mikroskop, so sieht man sofort bei dem Auffallen des Blutes auf den Objektträger verschiedene Veränderungen der Blutzellen vor sich gehen. Dieselben wurden zum großen Teil in einer früheren Arbeit beschrieben. Hier soll daher nur auf gewisse Punkte eingegangen werden. Im wesentlichen finden wir die gleichen Verhältnisse bei allen untersuchten Arthropoden. Gleich im Auffallen zerfließen eine Anzahl von Blutkörperchen. Eine mehr oder weniger weitgehende Verminderung der Oberflächenspannung vieler Zellen, und eine verschieden starke Mischung des Zellprotoplasmas mit der umgebenden Flüssigkeit findet statt.

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daß Zellen vorhanden waren als Granula, die hier und da zerstreut zu sehen sind. Da wir aber wissen, daß sehr viele Granula schwinden und zwar sehr oft mit großer Schnelligkeit, so haben wir vorläufig kein Mittel, um abzuschätzen, wie viele Blutkörperchen etwa spurlos aufgelöst wurden. In anderen Fällen tritt die Mischung zwischen umgebender Blutflüssigkeit und Zellen unter Vakuolenbildung ein. Nicht selten wird durch die Bewegung des Blutes eine ganze Zelle zu einem Faden ausgezogen. Im Anfang ist in einem solchen Falle der Kern zuweilen noch in der Mitte als eine kleine Anschwellung zu sehen. Granula schwinden auch hier großenteils; doch werden häufig manche in den Faden mit einbezogen und mögen hier für längere Zeit erhalten bleiben. In anderen Fällen verwandelt sich die Zelle, ohne sich direkt mit der umgebenden Flüssigkeit zu mischen, in eine formlose, körnchenhaltige Protoplasmamasse, die lang ausgezogen werden kann, ohne aber wohlentwickelte Fäden zu bilden. Man hat oft Schwierigkeit, diese Umwandlung nachzuweisen; auch hier weisen die übriggebliebenen Granula auf die stattgefundene Auflösung der Zellen hin. Aber auch die Konturen vieler Zellen bleiben nicht unverändert. Es treten nämlich allmählich aus den Zellen, welche im Tierkörper oval und flach sind, und Granula führen, die meist ganz in der Peripherie der Zelle gelegen sind, hyaline Pseudopodien aus. Diese können alle Formen annehmen und sich auch in Form von runden Tropfen um die Zelle bewegen. Zusatz verschiedener Lösungen zu dem Blut (Alkalien, destilliertes Wasser, Säuren), sind von Einfluß auf die Formveränderungen der Zelle. Tropfenbildung um die Zellen tritt besonders in verdünnten Alkalien und in destilliertem Wasser auf. Eine weitere Erscheinung, die bei manchen Zellen zu beobachten ist, besteht darin, daß die Peripherie der Zelle an einer Stelle reißt oder aufgelöst wird und daß sodann eine nicht immer gleich große Masse Zellprotoplasma mit Granula hervortritt. Dieser Vorgang wurde von Loewit sehr genau untersucht und als Plasmoschise bezeichnet. Wir sehen zuweilen anscheinend typische Pseudopodien an der Spitze oder nahe der Spitze ein Körnchen tragen; ein solcher ein Körnchen tragender Fortsatz tritt sehr schnell aus der Zelle aus. Die Plasmo-


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schise ist also wohl ein Vorgang, bei dem die peripherischen Teile der Zelle gesprengt oder aufgelöst werden und ein Teil des Zellprotoplasmas durch die so geschaffene Öffnung hervortritt, wobei meist einzelne Granula erhalten bleiben. Bei der Pseudopodienbildung findet wahrscheinlich ein etwas langsamerer Auflösungsprozeß des peripherischen Zellprotoplasmas unter Verminderung der Oberflächenspannung an dieser Stelle statt, wobei der Ausbreitung des Zellprotoplasmas ein Schwund der Granula parallel geht. Der oben erwähnte Fall stellt eine Zwischenstufe zwischen Plasmoschise und Pseudopodienbildung dar. Auch sonst geht die Auflösung (Verflüssigung) eines Teiles des Zellprotoplasmas und der Granula nicht parallel, falls die Mischung zwischen Zellprotoplasma und Blutflüssigkeit sehr schnell („explosiv") stattfindet. In diesem Falle bleiben sehr oft die Granula erhalten. Andrerseits kann, z. B. in schwachen Säuren, die Zellkontur sehr gut erhalten bleiben und die Granula können alle schwinden. Sonst entspricht die Auflösung der Granula meist einer Auflösung des Protoplasmas. Außerhalb der Zellen (nach stattgefundener Auflösung derselben) scheint die Existenz der erhalten gebliebenen Granula gesicherter zu sein, wie im Innern derselben.

Nachdem wir so eine Übersicht über die Haupttypen der anzutreffenden Zellveränderungen gegeben haben, sollen nun einige Angaben über den zeitlichen Verlauf der im Blute nach dem Ausfließen aus dem Körper stattfindenden Veränderungen gegeben werden. Hierbei soll auf manche der eben geschilderten Veränderungen keine weitere Rücksicht genommen werden; es sollen nur die leicht ins Auge fallenden Vorgänge hierbei berücksichtigt werden.

Beobachtet man schnell einen aus der Wunde auf den Objektträger auffallenden Blutstropfen unter dem Mikroskop, so schwimmen die im ersten Augenblick noch ovalen, granulären Blutzellen infolge der Bewegung des Tropfens frei umher. Schon im Laufe der ersten halben Minute fangen einzelne Blutkörperchen an sich abzurunden, und eine große Zahl der Granula ist bereits geschwunden. Nach Ablauf der ersten zwei Minuten sind fast alle Blutkörperchen rund. Bald nachdem die Abrundung begonnen hat, fangen auch einzelne Zellen an, Pseudopodien auszusenden, und am Ende der ersten drei Minuten haben fast alle

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Blutzellen Pseudopodien ausgestreckt. Soweit war die Bewegung der Blutkörperchen eine passive. Nun bleiben allmählich die Blutkörperchen, welche Pseudopodien ausgestreckt haben, sobald sie während der Bewegung zusammentreffen, aneinander kleben. Allmählich aber, und zwar schon im Laufe der ersten Minuten, werden auch Blutkörperchen, die noch keine Pseudopodien ausgestreckt haben, wie durch unsichtbare Fäden mit anderen, oft in beträchtlicher Entfernung sich befindlichen Zellen verbunden, wobei bei manchen Zellen eine vorausgegangene direkte Berührung als Ursache dieser Fadenbildung sich nicht nachweisen läßt. Nach zwei bis vier Minuten wird die Bewegung der Blutkörperchen immer beschränkter, benachbarte Zellen können sich nicht mehr unabhängig voneinander bewegen, und nach vier bis sechs Minuten steht der Blutstropfen auf dem Objektträger still, er fängt an eine Art Gelatine zu bilden. Unter dem Mikroskop sehen wir die untersten Lagen der Blutkörperchen auf dem Objektträger fixiert, die oberen Schichten sind noch mehr oder weniger beweglich. Gewöhnlich sind die benachbarten Blutkörper miteinander verbunden, und zwar besonders die in einer Ebene gelegenen Zellen. Bewegen wir nun ein Blutkörperchen mit einer Nadel, so bewegen sich viele andere mit. Doch können solche Verbindungsfäden reißen, und dann sieht man Zellen frei umherschwimmen, es ist also zu dieser Zeit nicht eine etwa alle Zellen einschließende Gallerte vorhanden, jedenfalls nicht eine Gallerte, welche die Bewegung einzelner Blutkörperchen verhindern würde. Doch kann bei Betrachtung mit dem bloßen Auge und bei Bewegung des Tropfens mit einer Nadel das Blut auf dem Objektträger zu dieser Zeit schon gelatinös erscheinen. Es bestehen hier wohl auch Unterschiede zwischen den verschiedenen Blutarten. Das Blut von Libinia (Spinnenkrebs) gerinnt weniger gelatinös, wie das von Limulus.

In den nächsten fünf Minuten nach Ausfließen des Blutes findet nun eine Annäherung der Zellen statt; die Zellen werden in Bündel und in Züge vereinigt, zwischen denen Flüssigkeit sichtbar wird. Zwischen den Zellen werden jetzt nicht selten lange Fasern deutlich. Gleichzeitig nun beginnt eine weitere Veränderung einzutreten. Es war schon oben darauf hingewiesen worden, daß die unteren Zellreihen am wenigsten beweglich

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sind. Dies beruht darauf, daß die in Berührung mit dem Glase tretenden Blutkörperchen an diesem festkleben. Wir können nun beobachten, wie die am Objektträger klebenden Zellen allmählich anfangen sich auszubreiten.

Bei den meisten Zellen unterscheiden wir im Anfang einen dunklen Innenkörper, welcher den Kern und die erhaltenen Granula einschließt, und eine hyaline exoplasmatische Zone, zu der die Pseudopodien gehören. Vielfach ist aber der endoplasmatische kontrahierte Teil von einer der Pseudopodiensubstanz ähnlichen hyalinen Zone vollständig umgeben. Im Laufe der nächsten Stunden findet nun eine allmähliche Vergrößerung der exoplasmatischen Zone statt. Der endoplasmatische Teil fängt nämlich auch an, sich auszubreiten, seine Körner werden kleiner und rücken auseinander; so nimmt das Protoplasma auch im Innenkörper allmählich eine hyaline Beschaffenheit an. Die Körner bewegen sich ebenfalls; sie fließen oft weit aus in die Pseudopodien und können zuletzt so gelagert sein, daß sie zwischen den Zellen zu liegen scheinen; dies kommt dadurch zu stande, daß Teile des Protoplasmas weit von dem Zellkern und dem Innenkörper wegfließen. Für lange Zeit ist in vielen Zellen der Innenkörper von der exoplasmatischen Zone durch eine scharfe Grenzlinie, eine Art Membran, getrennt. Diese Membran, innerhalb welcher der Zellkern liegt, schließt nur einen kleinen Teil des ursprünglichen Zellprotoplasmas ein. Gleichzeitig mit diesen Ausbreitungserscheinungen finden noch andere Veränderungen statt. Tropfen verschiedener Größe treten in dem Protoplasma auf; diese sind zuweilen ähnlich gelagert wie die Granula. Möglicherweise sind manche dieser Tropfen durch Verflüssigung der Granula entstanden, die meisten wohl durch Verflüssigungsvorgänge in dem Protoplasma. Bewegung des Blutes mit einer Nadel kann bewirken, daß diese Tropfen in eine größere Masse zusammenfließen, um später dann allmählich wieder sich in kleine Tropfen zu zerteilen und zuletzt zu schwinden. Wo die zuerst deutlichen Granula lagen, sieht man zuletzt nur ganz kleine Andeutungen von Körnchen, die später ebenfalls vollständig schwinden können. Wenn wir also sehen, daß diese Veränderungen zu einer Ausbreitung der Zellen

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führen, so finden daneben auch langsame, sowohl wie ruckweise Ortsveränderungen der Blutkörperchen selbst statt und zugleich ein fortwährender Wechsel der Pseudopodien; einzelne werden eingezogen, an anderer Stelle werden neue ausgestreckt. Zugleich treten oft Vakuolen im Innern des hyalin werdenden Protoplasmas auf, diese Vakuolen können ebenfalls ihre Gestalt verändern und eine größere kann sich zum Beispiel in zwei kleinere Vakuolen spalten. Alle diese Vorgänge führen nun dazu, daß die vorher durch Kontraktion des Fasersystems in Haufen und Strängen zusammengerückten Zellen sich sekundär weit ausbreiten, sodaß nach einer bis zwei Stunden, wenn die Verdunstung des Blutes verhindert wird, ein Netz von Zellen, die sich weit ausbreiten und deren pseudopodienartige Ausläufer fast alle in Verbindung miteinander stehen, vorhanden ist. An anderen Stellen führt der Vorgang dazu, daß im Anfang getrennte Zellhaufen nach etwa 20—30 Minuten zu einer Zellmasse vereinigt wurden. Ich habe mich nun bemüht, festzustellen, ob diese Ausbreitungserscheinungen nur an den in Berührung mit dem Glase befindlichen Zellen stattfinden, oder auch in den höheren Schichten. Man kann beobachten, daß allmählich durch ihre Schwere die oberen Zellballen ebenfalls zu Boden sinken und daß hier dann sofort diese Zellen festkleben; sogar solche Zellen, welche in den höheren Teilen der allmählich zu Boden sinkenden Zellballen gelegen sind, bleiben, sobald sie durch herabsinkende Pseudopodien mit dem Glas in Berührung kommen, mit diesem verbunden. Pseudopodien sind, wie oben erwähnt, sehr bald in fast allen Zellen vorhanden. Eine Ausbreitung jedoch, wie sie in den in Berührung mit dem Glase befindlichen Zellreihen stattfindet, konnte ich in den oberen Reihen nicht beobachten. Doch findet wohl auch hier eine Auflösung von Zellen statt, wie insbesondere in Sublimat fixierte Präparate zeigen. Außer diesen Zellhaufen nun sehen wir sehr bald Fäden der verschiedensten Dicke sichtbar werden. Allmählich werden die Zellen mehr und mehr durch Fäden ersetzt. Wenn wir Blut über Nacht stehen lassen und am nächsten Morgen untersuchen, finden wir sehr dicke Fadensysteme, teilweise durchsetzt mit den Resten von Zellen, insbesondere mit Zellkernen. Die Zeit, in der die Zellen schwinden, scheint in verschiedenen Fällen

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verschieden zu sein. Zuweilen waren schon nach wenigen Stunden im Blut von Libinia fast alle Zellen geschwunden und nur mehr ein Fadensystem vorhanden. Zuweilen waren die Zellreste noch am nächsten Tage vorhanden. Dieser schnelle Untergang der Zellen mag vielleicht teilweise durch die Anwesenheit von Bakterienprodukten bedingt sein. Aber wenn wir in Erwägung ziehen, daß schon in dem frischen Blute so eingreifende Zerfließungerscheinungen an dem Zellprotoplasma stattfinden, so wird wohl der Hauptfaktor in dem Charakter der Blutflüssigkeit gegeben sein.

Die Bildung von Fasern soll später im Zusammenhang besprochen werden. Hier soll nur erwähnt werden, daß auch in dem oben beschriebenen, durch Ausbreitung der Zellen entstehenden Netze Fasern sichtbar werden können, und zwar in dem exoplasmatischen Teil, ferner daß die Fäden Granula einschließen können, welche vorher aus dem Zellleib herausgeflossen sind. Bewegung bewirkt eine sehr ausgesprochene fibrilläre Umwandlung des Zellprotoplasmas. Sehr deutlich wird dieses in Stücken, die in Sublimat fixiert waren und dann nach Einbettung in Paraffin nach verschiedenen Methoden gefärbt werden. Hämatoxylin und Eosin, van Gieson, Mallorys Bindegewebsfärbung und Weigerts Fibrinfärbung wurden angewandt. Mit diesen Färbungen sehen wir deutlich, daß an manchen Stellen das ganze Protoplasma sich in Fasern auflöst. Vielfach wird jedoch nur der exoplasmatische Teil fibrillär. Der endoplasmatische Anteil mit dem Kern wird sehr oft von einer Membrau eingeschlossen, die sich ebenso färbt, wie die exoplasmatischen Fasern. Wir sehen in solchen Präparaten deutlich, daß das Protoplasma einer Zelle sich in viele Fibrillen auffasern kann. Diese, aus Zellen entstandenen Fasern färben sich nach Mallory wie Bindegewebe blau, nach Weigert leicht blau[1]). An vielen Stellen sind die Zellen regelmäßig nach Art epithelialer oder endothelialer Zellen aneinandergereiht, wobei sie nur durch die agglutinative Substanz ihres eigenen Zelleibes verbunden sind. An anderen Stellen sind diese Zellen in Intercellularsubstanz verwandelt, indem teilweise auf die oben beschriebene Weise fibrilläre

[1]) Für einige sehr schöne Präparate bin ich Herrn Dr. Movers in dem Mac Lean Hospital zu Danke verpflichtet.

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Strukturen aus dem ganzen Zellprotoplasma und insbesondere auch aus dem Exoplasma entstehen, oder auch indem die ganzen Zellen als solche zu Fasern ausgezogen werden. Über die Herkunft anderer Fasern soll später gesprochen werden. An verschiedenen Stellen haben wir Gewebe, das dem reticulären Bindegewebe ähnlich sieht: Multipolare Zellen sind durch lange Ausläufer netzförmig verbunden. Ein ähnliches Bild wird ja allein schon durch die früher beschriebene Ausbreitung der Zellen auf dem Objektträger hervorgebracht. Diese in Sublimat fixierten Präparate sind insofern wertvoll, als sie viele untergehenden Zellen und Kerne in verschiedenen Stadien der Auflösung fixieren. Wir können so feststellen, daß in der Tat in verschiedenen Zeiten nach dem Ausfließen des Blutes Zellen am Zugrundegehen sind und ganz verschwinden, ferner, daß oft Fasern an ihrer Stelle zurückbleiben, und daß die Kerne meist auf dem Wege der Karyorrhexis zu Grunde gehen.

P. Geddes führte die Koagulation des Blutes vieler wirbellosen Tiere auf die Bildung von Plasmodien zurück: Die Zellen strecken Pseudopodien aus, die sich mit Pseudopodien anderer Zellen verbinden; auch die Zellkörper der sich aneinanderlegenden Zellen selbst sollen verschmelzen. Der Vorgang wird von Geddes mit der bei Mycetozoen bekannten Plasmodienbildung verglichen. Demgegenüber weisen Pouchet und Cuénot darauf hin, daß eine eigentliche Verschmelzung nicht stattfindet, sondern daß die Zellgrenzen sichtbar bleiben. Es verhält sich nun in der Tat so, daß der endoplasmatische Teil der Zellen, der leicht als die ganze Zelle erscheinen könnte (und der ja auch durch eine Art Membran scharf begrenzt wird), bei der gewöhnlichen Gerinnung gewöhnlich nicht mit den benachbarten Zellen verschmilzt; daß hier lediglich die Membranen sich fest aneinander schließen; daß aber der exoplasmatische Teil mehrerer Zellen eine einzige Masse bildet, in der die den einzelnen Zellen zugehörigen Teile nicht voneinander unterschieden werden können.

Nachdem wir nun die gewöhnlichen Gerinnungserscheinungen wenigstens in den Hauptzügen beschrieben haben, sollen Untersuchungen über die experimentelle Beeinflussung der Gerinnung und insbesondere über das Verhalten der Blutkörperchen unter experimentell gesetzten Bedingungen mitgeteilt werden.

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Die Versuche wurden hauptsächlich an Limulus angestellt, da hier wegen des starken und schnellen Ausströmens des Blutes die sichersten Resultate erhalten wurden. Beim Hummer, den kleinen Krebsen, ebenso wie beim ganz kleinen Limulus läßt sich die Einwirkung der Salzlösungen aus den oben angegebenen Gründen nicht so deutlich nachweisen, selbst wenn das Blut nach Eintauchen in die Lösung in dieser direkt aufgefangen wurde. Ein gewisser schwächerer Einfluß der Salzlösungen war jedoch auch bei diesen Tieren vorhanden, sodaß wir annehmen können, daß der Unterschied zwischen Limulus und den anderen Tieren nur auf dem oben angegebenen Umstand der Verschiedenheit, der in der Schnelligkeit des Ausfließens besteht, beruht. Darauf weist auch die Tatsache, daß, wenn das Blut des Limulus im Herausströmen etwas verzögert wurde, auch hier die Gerinnung nicht mehr beeinflußt werden konnte. Aus diesen Verhältnissen geht hervor, daß eine genaue quantitative Bestimmung des Einwirkens der Salzlösungen nicht möglich ist, sondern daß es sich nur um untereinander annähernd vergleichbare Werte handeln kann. Die Versuche wurden in der Weise angestellt, daß 20—30 ccm der betreffenden Lösungen in Schalen gegossen, dann von der ventralen Seite aus das Tier geöffnet und nun ein kleiner Teil des ausfließenden Blutes in den in einer Reihe stehenden Schalen schnell aufgefangen wurde.

Folgende Salze wurden untersucht: Natrium carbonat, Natriumchlorid, Natriumnitrat, Natrumsulfat, Kaliumchlorid, Kaliumnitrat, Kaliumsulfat, Kaliumchlorid, Kaliumjodid, Ammoniumsulfat, Lithiumchlorid, Lithium carbonat, Calciumchlorid, Bariumchlorid, Strontiumchlorid, Magnesiumsulfat, Kaliumoxalat, Natriumcitrat, Kalium- und Natriumtartrat, Aluminiumammoniumsulfat, Ferrocyankalium, Cyankalium, Kupfersulfat.

Es kommen nun von vornherein nur die ganz bis halbgesättigten Lösungen in Betracht. Ausgeschieden werden von diesen Untersuchungen Kaliumjodid, Ammoniumsulfat, Lithiumchlorid, Calcium-, Barium-, Strontiumchlorid, Cyankalium und Kupfersulfat. In diesen Lösungen nämlich entstand auf Zufügen des Blutes ein so starker Niederschlag, daß eine Untersuchung der Gerinnung und des Verhaltens der Blutkörperchen aus-



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weder garnicht sichtbar oder doch nicht so bedeutend, daß die Einwirkung auf die Gerinnung dadurch verdunkelt worden wäre. Alle übrigen Substanzen haben einen hemmenden Einfluß auf die Gerinnung des Limulusblutes, doch ist derselbe nicht gleich stark bei allen diesen Substanzen. Vergleichen wir gesättigte Lösungen, so haben die genannten Natriumsalze einen stärkeren Einfluß, wie die Kaliumsalze. Ferrocyankalium hat nur einen geringen hemmenden Einfluß. Aluminiumammoniumsulfat hat einen ausgesprochen hemmenden Einfluß. Auch Natriumcarbonat hat einen stark hemmenden Einfluß, obwohl es nicht neutral reagiert. Doch kam es hier vor, daß nach einiger Zeit in der Natriumcarbonat-Lösung sich das Blut zu einer syrupösen Masse löste.

So war in einem Versuch in der gesättigten $Na_2 CO_3$-Lösung nach 15 Minuten langem, ruhigem Stehen keine Koagulation vorhanden. Noch nach 20 Minuten trat nach Schütteln keine Koagulation ein, doch war das Blut jetzt schon nicht mehr so leichtflüssig. Nach 50 Minuten hat das Blut eine syrupartige Konsistenz angenommen. Die Blutkörperchen lagen in einem Schleim eingebettet, aber dieser war in der gesamten Flüssigkeit verteilt und hinderte die Blutkörperchen nicht an freier Bewegung. In anderen Fällen jedoch hinderte die Natriumcarbonat-Lösung die Gerinnung viel länger.

Gesättigte Lithiumcarbonat-Lösungen hatten keine ausgesprochen hemmenden Wirkungen. Doch zeigte sich ein starker Einfluß dieser Lösungen auf die Zellen darin, daß die Zellgranula unter dem Einfluß von Lithiumcarbonat in großer Zahl erhalten blieben. Magnesiumsulfat hatte einen ausgesprochenen hemmenden Einfluß auf die Gerinnung, ebenso Natriumnitrat. In Natriumnitrat war gewöhnlich kein nennenswerter Niederschlag zu sehen. Auch in Magnesiumsulfat und in Aluminiumammoniumsulfat war kaum ein Niederschlag vorhanden. Die Menge des Niederschlags war im übrigen außerordentlich wechselnd. Jedenfalls konnte keinerlei Beziehung zwischen der gerinnungshemmendnn Eigenschaft dieser Salze und einer etwaigen Fällung des Fibrinogens festgestellt werden. Eine besondere Aufmerksamkeit wurde den Salzen zugewandt, welche eine spezifische


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Wirkung auf das Blut vieler Wirbeltiere haben, nämlich dem Kaliumoxalat und den Citraten. Diese wirken nun beide ebenso wie das Tartrat nur unter denselben Bedingungen, wie die übrigen Salze, nämlich in gesättigter bis halbgesättigter Lösung. In schwächerer Lösung sind sie ganz unwirksam. Auch wenn 10—15 ccm 4,5 pCt. Kaliumoxalat-Lösung in einen Limulus injiziert wurden und 10 Minuten später das Blut entzogen wurde, fand Gerinnung des Blutes statt. Wir können daraus schließen, daß die Wirkung des Oxalats nicht auf seiner Calcium fällenden Eigenschaft beruht, sondern daß es nur wie alle anderen Neutralsalze wirkt.

Es hängt nun in allen diesen Fällen der Grad der Gerinnungshemmung non gewissen Nebenumständen ab. Ist die Menge des in die Lösung einfließenden Blutes zu groß, so gerinnt dasselbe, besonders das in der Mitte des Blutstromes befindliche Blut, das nicht sofort mit der Lösung in Berührung kommt. Aber auch sonst ist die Gerinnungshemmung nicht immer eine absolute. An den Seiten, in Berührung mit den Glaswänden des Gefäßes, mag ein kleiner Teil des Blutes gerinnen; Bewegung hat häufig, besonders im Anfang, einen gewissen die Gerinnung beschleunigenden Einfluß. Soweit ich bisher sehen konnte, ist dieser Einfluß der Bewegung in diesen Lösungen nicht immer gleich deutlich und verschwindet, nachdem das Blut einige Zeit mit der Lösung vermischt war. Die Gerinnungshemmung ist aber in den meisten Lösungen zeitlich beschränkt. Nach einigen Stunden oder einem Tage findet meist doch etwas Fadenbildung in den Lösungen statt; wir finden in dieser Zeit am Boden des Gefäßes einen dünnen Belag, der aus aneinanderklebenden Blutkörperchen mit mehr oder weniger Fäden vermischt, besteht.

Macht man nun entsprechende Versuche mit verdünnten Lösungen, so bemerkt man, daß bereits Zusatz des halben Volums Wasser zu der gesättigten Salzlösung die Wirkung der Salzlösungen schwächt, noch mehr geschieht dies bei Zusatz eines gleichen Volums Wasser.

Als Beispiele mögen einzelne Versuche im besonderen angeführt werden; nicht jeder Versuch läßt in allen Einzelheiten die oben angegebenen Gesetzmäßigkeiten erkennen, hierzu ist ein genauer Vergleich einer Reihe von Versuchen nötig.

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In a) 20 ccm Kaliumoxalat-Lösung, b) 15 ccm derselben Lösung + 5 ccm destil. Wasser, c) 15 ccm derselben Lösung + 15 ccmm dest. Wasser.

In a bildet sich Präcipitat in den ersten Minuten, in b sehr wenig, in c kein deutliches Präcipitat. In allen ausgesprochene Gerinnungshemmung.

d, e, f sind die entsprechenden Lösungen von Natrium-Kalium-Tartrat. Lösung d und e zeigen kaum Gerinnung, Lösung f zeigt bald etwas Gerinnung, aber ein Teil des Blutes gerinnt hier nicht; Natrium-Kalium-Tartrat hat also einen gerinnungshemmenden Einfluß. In den Oxalat- und Tartratlösungen sind im allgemeinen die Zellen oval, wohlerhalten, meist mit Granula versehen. In Lösung d bildet sich bald ein Präcipitat, in e und f kaum Niederschlag.

g, h, i sind die entsprechenden Lösungen von Natriumsulfat, j, k, l von Kaliumsulfat. In j bildet sich etwas mehr Gerinnung wie in g (in j bildet sich ein Spinnwebennetz von Coagulum; daneben sind Häufchen agglutinierter Zellen makroskopisch sichtbar). In den Lösungen i, k und l mehr Gerinnung, Zellen in Häufchen vereinigt. In den Sulfatlösungen werden die Zellen im Lauf der ersten Stunde rund, senden sodann stachlige Pseudopodien aus, in Lösungen i und l mehr Pseudopodien ausgestreckt, wie in den anderen Lösungen. Nach 1¼ Stunden ist der gerinnungshemmende Einfluß der beiden verdünnten Tartratlösungen deutlich, in e kaum Niederschlag vorhanden, sehr wenig Gerinnung. In den Oxalatlösungen ist jetzt am Boden der Schalen eine fibrinartige, fädige Masse ausgebreitet. In den Kaliumsulfatlösungen ist zu dieser Zeit das Blut geronnen, in der stärkeren Natriumsulfat-Lösung nicht.

Ein Versuch, in dem insbesondere die Wirkung von Kaliumchlorid und Natriumchlorid verglichen wurde: Limulusblut wurde in der üblichen Weise entzogen und aufgefangen (etwa 5—10 ccm), in a) 20 ccm gesättigter Natriumnitrat-Lösungen. b) 15 ccm derselben Lösung + 5 ccm dest. Wasser. c) 15 ccm derselben Lösung + 15 ccm dest. Wasser. d) 20 ccm gesättigter Natriumchlorid-Lösung. e) 35 ccm derselben Lösung + 7 ccm dest. Wasser, f) 15 ccm derselben Lösung + 15 ccm dest. Wasser. g) 20 ccm


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Kaliumchlorid. h) 15 ccm derselben Lösung + 7 ccm dest. Wasser. i) 15 ccm derselben Lösung + 15 ccm dest. Wasser. Im Laufe der ersten Minuten: in a Gerinnung am Rande des Glases, der größere Teil ungeronnen. b Halb als Spinnwebennetz, halb in Flockenform geronnen (Gerinnungen in Flocken stellt einen geringeren Grad der Gerinnung dar, da hier die einzelnen geronnen Teile, die Flocken, unter sich unverbunden bleiben, während bei der Bildung eines spinngewebenartigen Netzes in der Flüssigkeit alle Teile verbunden sind). c Größtenteils als Spinnwebennetz geronnen. d Erst nach einigen Stunden am Boden des Glases kleinflockige Gerinnung. e Gleich im Anfang ein wenig spinnwebenartige Gerinnung, nach einigen Stunden am Boden wie Spinnwebennetz geronnen; jedoch ein Teil ungeronnen. f Schon im Anfang mehr spinnwebenartige Gerinnung, wie in e, nach einigen Stunden das ursprüngliche Gerinnsel in der Flüssigkeit schwebend, der Rest der Zellen am Boden. g Gleich im Anfang mehr Gerinnung wie in d, fast so viel wie in e. h zeigt sofort mehr Gerinnung wie g. Nach einigen Stunden etwas in der Flüssigkeit schwebendes Gerinnsel, der größte Teil des Blutes am Boden festklebend. i Gleich Gerinnung.

Durch frühere Versuche war bereits festgestellt worden, daß Kaliumnitrat, wenn es überhaupt irgend einen Einfluß, nur einen sehr schwachen hat. In Natriumnitrat hingegen gelang es oft, das Blut tagelang großenteils ungeronnen zu erhalten. Ebenso in Magnesiumsulfat-Lösungen wochenlang. Vielfach aber kleben auch in diesen Lösungen nach Ablauf von zwei oder mehr Stunden viele Zellen am Boden des Gefäßes, ohne das zwischen ihnen Fibrin zu sehen wäre. Die Zellen können gewöhnlich leicht wieder durch eine Nadel voneinander getrennt werden.

Wir sehen also, daß eine größere Anzahl von Salzen eine gerinnungshemmende Wirkung haben. Halliburton fand Natriumchlorid und Magnesiumsulfat wirksam, fand aber Natriumsulfat wirkungslos. Weiterhin sehen wir, daß das oxalsaure Salz nur in ebenso starker Konzentration wirkt, wie die die übrigen Salze. Wir können daraus schließen, daß die Wirkung des Oxalates wahrscheinlich nicht auf der Präcipitation von Calcium beruht. Auch wenn Oxalsäurelösungen (20—30 ccm einer 4 pCt. Lösung)

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dem Tiere eingespritzt wurden, bevor das Blut entnommen wurde, wurde die Gerinnung des Blutes nicht aufgehoben. Dieser Versuch war nötig, weil die Gerinnung so schnell außerhalb des Körpers eintritt, und deshalb der Einwand gemacht werden könnte, daß auch schwächere Oxalatlösungen wirksam wären, wenn sie früher mit Blut vermischt würden.[1]) Wie wirken diese Lösungen nun auf die Blutkörperchen ein? Sie bewahren dieselben großenteils vor den oben beschriebenen Veränderungen, d. h. sie verhindern nicht ganz, aber doch in sehr hohem Grade das Austreten von Protoplasma aus den Zellen, die Bildung eines exoplasmatischen Teiles um den mit einer Membran umgebenen endoplasmatischen, die Plasmoschise, das Vermischen ganzer Zellen mit der umgebenden Flüssigkeit, das Ausziehen einzelner Zellen zu Fäden. Die Blutkörperchen bleiben jedoch nicht ganz konserviert. Ein Teil der Granula geht immer verloren. Sehr stark präservierend auf die Granula wirkt, wie schon oben angegeben, das Lithiumcarbonat; aber ein Teil der Granula bleibt gewöhnlich erhalten; die ovale Form bleibt ebenfalls mehr oder weniger erhalten. Doch ließ sich hier ein interessanter Unterschied feststellen. Die Nitrate und Chloride und auch Natrium und Lithiumcarbonat erhielten mehr oder weniger die ovale Form, in Natrium- und Kaliumsulfat aber wurden die Zellen im Verlauf der ersten Stunde rund, und streckten dann Pseudopodien aus, obwohl ja auch Natriumsulfat einen entschieden gerinnungshemmenden Einfluß hat. Kaliumsulfat verändert die Zellen stärker wie Natriumsulfat, wie es ja auch weniger gerinnungshemmend wirkt. Wenn man die oben angegebenen Verdünnungen der Salzlösungen anwendet, so werden entsprechend der angewandten Verdünnung die Veränderung der Blutkörperchen (Austreten von Protoplasma, Pseudopodien) stärker. Ferner kleben in den konzentrierten Salzlösungen auch die Blutkörperchen nur wenig oder garnicht zusammen. Dies ist jedoch verschieden,

[1]) Kürzlich untersuchte auch Botazzi, wie ich aus einem in dem Centralblatt f. Physiologie, 1902, No. 13 erschienenen Autoreferat ersehe, die Wirkung von Oxalaten und Pepton auf das Crustaceenblut. Er fand, wie ich, daß Oxalate nur in sehr starker Konzentration wirksam sind. Ob er die Oxalatwirkung der Kalkpräcipitation zuschreibt oder nicht, ist aus dem Referat nicht zu ersehen.

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entsprechend der größeren oder geringeren Gerinnungshemmung der Lösungen. Viele Blutkörperchen mögen ganz frei umherschwimmen, viele andere aber können, wenn die Gerinnungshemmung nicht vollständig war, Häufchen bilden. Wir erwähnten schon oben, daß das Blut ganz kleiner Limuli oder Hummerblut und das Blut kleinerer Krebse so langsam herausfließt, daß nur eine geringe Einwirkung der Salzlösungen stattfindet. In einem solchen Falle nun konnte ich unter dem Mikroskop beobachten, daß die Zellen der Libinia, in gesättigter Magnesiumsulfat-Lösung auf dem Objektträger aufgefangen, allmählich sich abrundeten, und Pseudopodien ausstreckten; sie schwammen währenddem frei umher. Wo sie aber einander trafen, blieben sie mit ihren Pseudopodien kleben, und so kam die Bildung von Zellflocken zu stande, auf Grund der Klebrigkeit des Protoplasmas.

Da die Salzlösungen die Veränderungen an den Blutkörperchen verlangsamen oder ganz aufheben, so sind die in Salzlösungen befindlichen Blutkörperchen geeignet zu Beobachtungen über den Einfluß von mechanischen und anderen Einwirkungen auf diese Zellen. Wir können nun beobachten, daß die hyaline Ausbreitung der Blutzellen, wie sie oben am normalen Blute außerhalb des Körpers beschrieben wurde, hier nicht stattfindet. Die Blutkörperchen, die am Boden der Schale sich ansammeln und dort locker zusammenkleben, oder auch in distinkten kleinen Flocken lagern, zeigen dieses Phänomen nicht. Hingegen sind die Blutkörperchen nicht merkbar verändert in ihrem Verhalten gegen mechanische Einflüsse. Schon wenn wir das Salzblut auf den Objektträger bringen, können wir beobachten, daß eine Anzahl der Zellen explodieren, und sich am Boden weit ausbreiten. (Das ist ein von der sekundären hyalinen Ausbreitung verschiedener Vorgang.) Eine Reihe von Zellen kann sich in diesem Zustand flächenhaft oder in Zügen aneinanderlegen und, viele der Granula können erhalten bleiben. Noch besser können wir diese „Explosion“ infolge mechanischer Beeinflussung durch Auflegen eines zweiten Objektträgers auf den ersten hervorbringen. Besonders in dem Lithiumcarbonatblut, in dem die Granula gut erhalten bleiben, konnte nach Auflegen eines zweiten Objektträgers beobachtet werden, daß eine sehr große Anzahl von Blutkörperchen gesprengt wurden. Das Protoplasma mit den Granula trat

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aus und bildete eine diffuse Masse, wie leicht an der Verteilung und der Bewegung der Granula zu sehen war. Es war nun interessant, daß nahe der Stelle, wo die „Explosion“ stattfand, das Protoplasma noch eine zähere Masse bildete, weiter weg aber das Protoplasma wohl durch seine Mischung mit der umgebender Flüssigkeit viel leichtflüssiger geworden war, und daß hier die Granula sich überall in der Flüssigkeit verteilten. In Alaunblut konnte ich sehr deutlich beobachten, wie Druck eine Zelle zum Bersten bringen kann; ein Teil des Protoplasmas tritt aus. Die Zelle schließt sich aber sofort wieder, und dann glaubt man eine unversehrte, wenn auch etwas kleinere Zelle vor sich zu haben, während die Zelle in Wirklichkeit einen bedeutenden Teil ihres Protoplasmas an die Außenflüssigkeit abgegeben hat. Ferner treten hierbei einzelne Granula aus und solche Körner, die von der Zelle weit entfernt sind, sind durch unsichtbare Fäden des ausgetretenen Zellprotoplasmas mit der Zelle verbunden. Wird ein peripherisch gelegenes, ausgetretenes Granulum mit einer Nadel von der Zelle weggezogen, so werden alle anderen ausgetretenen Granula in derselben Richtung von der Zelle wegbewegt.[1]) Im Anfang können also die Zellen in Salzlösungen ihre physikalischen Eigenschaften mehr oder weniger beibehalten. Nach einiger Zeit aber schrumpfen sie etwas zusammen und werden wahrscheinlich weniger dehnbar. Doch konnte noch nach 16 Stunden in dem Alaunblut beobachtet werden, daß durch geringe mechanische Einflüsse Zellen zu sehr langen Fäden ausgezogen werden können. Da dasselbe mechanische Moment, welches die Zellen auszieht, sich gewöhnlich über ein weites Gebiet der Flüssigkeit erstreckt, so ordnet es auch mehrere Zellen in der Längsrichtung aneinander. Die Zellen verlieren dabei oft, aber nicht immer, die Granula. Die Kerne sind im Anfang noch in der Mitte sichtbar. Die Zellfäden werden nach der Peripherie zu dünner. Eine einzelne Zelle kann außerordentlich stark ausgezogen werden.

Verdünnen wir solche Blutsalzlösungen mit einem Überschuß von destilliertem Wasser, so verschwinden meist die noch er-

[1]) Bei ähnlichen Versuchen mit Blut in Natriumsulfat-Lösungen fand ich, daß an infolge von Stoß aus Zellen ausgetretenem Protoplasma andere Zellen kleben, und wenn auch in geringem Umfang ähnliche Bilder zustande kommen, wie bei der Gerinnung des Blutes.

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halten gebliebenen Granula der Zellen, aber im übrigen bleiben viele Zellen scheinbar unverändert. Oft, aber nicht immer, tritt nun noch Faserbildung in dem nachträglich verdünnten Salzblut auf. Auch wenn wir Glycerin oder gesättigte Rohrzuckerlösungen zu diesen Salzlösungen zusetzen, schwinden die Granula und Zellen gehen dabei zu Grunde. Einigemal trat dies auch ein nach Zusatz von Rohrzucker in Substanz zu der Lösung. In anderen Fällen hatte aber ein solcher Zusatz von einem Stückchen Zucker keinen Einfluß.

Da bei Zusatz von konzentrierten Lösungen der Erdalkalichloride auf das Blut ein so starker Niederschlag entsteht, daß die spezifische Einwirkung dieser Lösungen nicht sicher festzustellen ist, so versuchte ich durch geringen Zusatz einer konzentrierten Lösung eines solchen Salzes zu in Natriumnitrat-Lösung aufgefangenem Blut über die Wirkung dieser Lösungen Aufschluß zu gewinnen. Dabei wurde so verfahren, daß eine geringe Menge des in Natriumnitrat-Lösung präservierten Blutes auf einen Objektträger getropft wurde. Neben diese Natriumnitrat-Lösung wurde ein Tropfen Strontiumchlorid gebracht. Der Niederschlag bei Berührung beider Tropfen trat erst spät ein und konnte das Resultat nicht verdunkeln. Bei Berührung beider Tropfen traten Gerinnungserscheinungen sofort auf, Zellen gingen zu Grunde und runde, von Zellen gebildete Protoplasmatropfen schwimmen umher. Fäden wurden bald zwischen vielen Zellen sichtbar. Ohne daß die Möglichkeit einer anderen Entstehungsart eines Teiles der Fasern geleugnet werden soll, konnte an vielen Stellen die Entstehung dieser Fäden auf folgende Weisen beobachtet werden: 1. Zellen werden ausgezogen; hierbei bleibt der Kern oft als eine Anschwellung im Centrum sichtbar. 2. Zellen explodieren, ihr Protoplasma mit den eingelagerten Granula breitet sich flächenförmig aus. Unter geeigneten mechanischen Bedingungen verwandelt sich dieses Protoplasma in Fäden. 3. Durch Plasmoschise werden Zellen zum Teil zerstört; ein Teil ihres Protoplasmas tritt aus; dieses wird durch die Bewegung der Zellen zu Fäden ausgezogen, an denen andere Zellen adhärieren. So werden die früher in der Natriumnitrat-Lösung frei beweglichen Zellen unter dem Einfluß des Strontiumchlorids unbeweglich und größere Zellballen entstehen. In der Mitte dieser Ballen

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werden größere Fasern sichtbar. Man kann sehen, wie einzelne Zellen in diesen Ballen aufgelöst werden. Es ist interessant, daß die Fasern im Allgemeinen in der Richtung liegen, in der das Strontiumchlorid in die Blutlösung diffundiert. Die Flüssigkeit bestimmt durch ihre mechanische Einwirkung die Richtung der Fasern. Es kann unter den verschiedensten Bedingungen beobachtet werden, daß mechanische Einflüsse ganz leichter Art nicht nur die Lage schon gebildeter Fibrinfasern bestimmen, sondern auch die Richtung sich erst bildender Fasern. Wir sehen also, daß Strontiumchlorid und, wie ich aus andern Beobachtungen schließen kann, Calciumchlorid und Bariumchlorid die gerinnungshemmende, zellerhaltende Funktion anderer Salzlösungen nicht haben. In Calciumchlorid kann von den Blutkörperchen in kurzer Zeit nur mehr eine Schale zurückbleiben. Viele andere Blutzellen werden hyalin. Im Hummerblut treten in vielen dieser Salzlösungen stärkere Niederschläge ein, wie im Limulusblut. Doch haben dieselben Salze auch hier meist dieselbe präservierende Wirkung auf die Zellen. Sie verhindern gewöhnlich nicht die Zusammenballung derselben zu kleinen Haufen, haben aber doch oft einen schwach hemmenden Einfluß auf den Grad der Agglutination, und die gelatinöse Gerinnung des Hummerblutes unterbleibt unter ihrem Einfluß. Dieselben Unterschiede bestehen in der Wirkung von Chloriden und Sulfaten auf die Form der Zellen im Hummerblut wie bei Limulusblut.

Einwirkung einiger organischer Substanzen.

Es wurde weiterhin die Einwirkung einiger organischer Substanzen auf die Gerinnung untersucht:

1. Pyrrhol, Hydrochinon und Resorcin üben alle, ohne die Gerinnung ganz aufzuheben, a) einen hemmenden Einfluß aus, sodaß ein Teil des Blutes ungeronnen bleibt oder die Gerinnung wenigstens verzögert wird.

Man kann zuweilen beobachten, daß einige Zeit nach dem Auffangen des Blutes dasselbe ungeronnen bleibt, dann aber infolge von Bewegung der Flüssigkeit die Blutkörperchen in kleinen Häufchen zusammenkleben und nun zu Boden sinken; in anderen Fällen bleibt die Mehrzahl der Blutkörperchen einige Zeit frei, dann

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aber sinken dieselben zu Boden und kleben zusammen. Beim Resorcin wurde beobachtet, daß es eine ganz kurze Zeit die Gerinnung aufzuheben schien, dann jedoch das Blut vom Limulus in Form einer gelatinösen Masse gerann.

Es kommen also hier Variationen vor.

b) die Blutkörperchen werden mehr oder weniger präserviert. Sie bleiben gewöhnlich groß und behalten entweder ihre ovale Form oder werden rund. Viele derselben behalten einen großen Teil ihrer Granula. Die Blutkörperchen bleiben weich. Diese Beobachtungen konnten bei dem Blute aller untersuchten Tiere gemacht werden.

In den kleineren Arthropoden ist auch hier die Einwirkung oft weniger ausgesprochen; wir mögen z. B. bei Libinia nach kurzer Zeit viele Blutkörperchen mit Pseudopodien versehen finden. Es wurden zu diesen Versuchen sowohl Lösungen der betr. Substanzen in Seewasser, wie in destilliertem Wasser verwendet. Pyrrhol ist nur sehr wenig löslich; doch genügte oft die geringe in Lösung gehende Menge, um einen deutlichen Einfluß auszuüben. Von Hydrochinon und Resorcin wurden 2—6 pCt. Lösungen verwandt. In der Mehrzahl der Fälle wirkte Hydrochinon am besten präservierend auf die Zellen. Pyrrhol wirkte zuweilen nicht merklich ein, zuweilen jedoch deutlich. Ein Niederschlag trat früher oder später ein, jedoch nicht schnell genug, um die spezifische Einwirkung undeutlich zu machen. Das Blut wurde bei diesen Versuchen, wie bei den früheren in Schalen oder auf Objektträgern in der betreffenden Flüssigkeit direkt aufgefangen.

Keine dieser Substanzen war im stande, die nach kurzer Zeit im Hummerblut beim Auffangen auf dem Objektträger stattfindende gelatinöse Umwandlung (zweite Gerinnung) zu verhindern; in stärkeren Lösungen unterblieb die hyaline Ausbreitung der Zellen. In ganz schwachen Resorcinlösungen jedoch und in Pyrrhollösungen konnte dieselbe beobachtet werden. Doch sollen hierüber noch weitere Versuche angestellt werden. Da die Zellen unter der Einwirkung dieser Substanzen gewöhnlich weichelastisch bleiben, so konnten auch hier durch mechanische Einwirkungen die Zellen verändert werden. Zellen können verletzt werden, wobei Protoplasma austritt; Granula können mechanisch

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oder durch Auflegen eines zweiten Objektträgers können Zellen, die frei waren, zur Agglutination veranlaßt werden. Diese agglutinierten Zellhaufen können zuweilen alle Granula verlieren, sodaß dann Häufchen granulöser runder Zellen vorliegen, wie ich sie schon in meiner früheren Arbeit als charakteristisch für schwache Säurewirkung beschrieben habe.

In 5 pCt. Karbolsäure aufgefangen, bleiben die Blutkörperchen nicht vor den beschriebenen Veränderungen ganz bewahrt; auch wird die Gerinnung nicht verhindert.

Weiterhin wurde die Einwirkung einiger Alkaloide, wie Atropin, Pilocarpin, ferner die Wirkung von Adrenalin[1]) geprüft. Es wurden hierzu zum Teil Lösungen der Substanzen in einer Konzentration von 1 : 300 in Seewasser oder in destilliertem Wasser benutzt, ferner wurde eine Adrenalinlösung von 1 : 1000 unter Zusatz von Chloreton versucht. Die letztgenannte Lösung gab einen starken Niederschlag mit dem Blut. Hierbei fand zuweilen die Gerinnung in einzelnen Flocken oder in Form eines zusammenhängenden Fadensystems statt. Also war auch hierbei wohl eine geringe Gerinnungshemmung vorhanden. Auch in dem reinen Adrenalin (1 : 300) fand eine Gerinnungshemmung statt, wenn der Blutstropfen schnell auf den mit der Lösung bedeckten Objektträger auffiel. Hier werden zuweilen nur Flocken statt eines zusammenhängenden Netzes gebildet, es lag dann also eine Gerinnungshemmung vor. Kam der Tropfen etwas langsamer heraus, wie das besonders beim Hummer und den Krebsen gewöhnlich der Fall ist, so gerann der größte Teil des Blutes in der gewöhnlichen Weise. Aber auch beim Auffangen des Blutes in Schalen, welche die Lösungen von Pilocarpin, Adrenalin, Atropin (1 : 300) enthalten, trat meist eine spinnwebige Gerinnung ein. Fängt man das Blut auf dem Objektträger in diesen Lösungen auf, so treten gewöhnlich am Rande des Bluttropfens, da wo die Lösung leicht einwirken konnte, gewisse Veränderungen der Zellen ein, die von Interesse sind. Die Zellen wurden gewöhnlich sehr groß, und häufig kubisch. Doch kamen auch runde und

[1]) Diese Substanzen wurden mir in freundlicher Weise von Herrn Dr. McClintock, Leiter des biologischen Laboratoriums von Parke, Davis u. Co. zur Verfügung gestellt.


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etwas kleinere Zellen vor. Die normalen Granula verschwanden meist. Man sah entweder granuläre Zellen oder Zellen, welche größere Tropfen enthielten, die in der Peripherie der Zellen dicht unter einer äußeren Schicht lagen; diese äußere Schicht, die das Aussehen einer Membran hatte, bestand wohl aus derselben Substanz, wie das übrige Zellprotoplasma, unterschied sich aber wohl dadurch von diesem, daß sie sich unter dem Einfluß einer größeren Oberflächenspannung befand; nicht selten wurde jedoch auch der peripherische Teil der Zelle selbst in distinkte Tropfen aufgelöst. Zuweilen erschienen auch im Innern der Zelle deutliche Vakuolen, die den Kern auf eine Seite drückten. Manche Zellen waren völlig undurchsichtig. Das war der Fall in Zellen, deren Protoplasma zum größten Teil in dicht aneinandergelagerte Tropfen umgewandelt war. Diese Zellen selbst waren gewöhnlich rund und kontrahiert. Zusatz von Alkali löste die intracellulären Tropfen auf. Die Größe der Zellen in diesen Lösungen und die durch diese Lösungen bewirkte, wenn auch nur schwache Gerinnungshemmung, machte die Blutkörperchen in Adrenalin zu Beobachtungen sehr geeignet. Bemerkenswert ist, daß die Zellen hier häufig in längeren oder kürzeren Reihen zusammenkleben. Man kann hier deutlich sehen, daß die Zellen, ohne von irgend einer gelatinösen Masse umhüllt zu sein, mit ihrem eigenen Protoplasma zusammenkleben in Form von Reihen, die eine vollständig epitheliale Struktur zeigen. Dieses Zusammenkleben konnte so weit gehen, daß die Grenzen zwischen zwei aneinanderliegenden Zellen ganz schwinden konnten und die beiden Zellen ein Ganzes bildeten. Die erste Zelle einer solchen Reihe mochte am Objektträger festkleben und die anderen mit dieser verbundenen Zellen konnten vollständig frei in der Flüssigkeit schweben. Zuweilen waren auch mehrere Reihen in dieser Weise zu kleineren oder größeren epithel- oder endothelartigen Zellplatten vereinigt. Die Beschaffenheit des Protoplasmas solcher Zellen war dem noch nicht kontrahierten Blutfaserstoff durchaus ähnlich. Das Protoplasma war klebrig. Es klebte am Objektträger fest, und andere Blutzellen, die in Berührung mit dem Protoplasma solcher fixierten Blutkörperchen kamen, klebten ebenfalls fest. Das Protoplasma war weich und

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war elastisch. Zellen, die mit einer Nadel ausgezogen wurden, schnellten beim Nachlassen des Zuges in ihrere Lage zurück. Über weitere Versuche, die mit diesen Zellen angestellt wurden, soll nachher berichtet werden. Hier konnte in anderen Fällen wieder sehr deutlich beobachtet werden, daß zuweilen Protoplasma mit den Granula aus den Zellen heraustrat und daß an diesem ausgetretenen Protoplasma andere Zellen hängen blieben. Hier konnte auch beobachtet werden, daß, wenn man die überschüssige Flüssigkeit von dem Objektträger abfließen ließ, diese geringe mechanische Einwirkung genügend war, um viele Zellen in lange Fäden auszuziehen, die alle in derselben Richtung angeordnet waren. Schon während des Auffallens des Tropfens konnten viele Zellen eine ähnliche Umwandlung zu Fasern erleiden.

Der Kern war auch weich und dehnbar. Es konnte beobachtet werden, daß der Kern in der Mitte einer solchen Faser sehr lang ausgezogen wurde, sodaß er bald im Innern der Faser ganz unsichtbar wurde. Wahrscheinlich unterliegt er ebenfalls später Auflösungsprozessen, wie das in anderen Fällen schon oben an den gefärbten Präparaten gezeigt werden konnte. Zuweilen blieb der Kern als eine kleine Kugel im Centrum der Faser zurück, indem er dort eine kleine Anschwellung bewirkte. Die Vakuolen, die unter dem Einfluß dieser Substanzen entstehen, können ebenfalls langgestreckt werden. Die Blutzellen auf dem Objektträger können auch Pseudopodien ausstrecken, falls die Wirkung des Adrenalins nicht zu stark war; am Rande des Blutstropfens pflegte dies nicht der Fall zu sein, da hier die volle Wirkung des Adrenalins zur Geltung kam. Üben wir auf solche Zellen einen Zug aus, so ordnen sich die erhaltenen Granula in Reihen an, und eine fibrilläre Struktur der Zellen wird deutlich. Adrenalin zeigt die bisher beschriebene Einwirkung deutlicher wie Pilocarpin und Atropin. Doch ist sie am Rande des Blutes auch hier vorhanden.

Die Wirkung von Adrenalin ist aber insofern spezifisch, als es (in geringem Maße) gerinnungshemmend wirkt. Im übrigen sind die beschriebenen Veränderungen nur der Wirkung von Wasser


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oder sehr verdünntem Alkali zuzuschreiben, Wirkungen, die zum Teil wohl lediglich auf osmotischen Vorgängen beruhen und zu einem Aufschwellen und zu tropfenförmigen Auflösungsvorgängen in der Zelle führen. Aber gerade die Kombination der Wasserwirkung mit einer gewissen Gerinnungshemmung macht Adrenalin sehr geeignet zu Versuchen mit den Blutzellen. Die oben beschriebene Hydrochinonlösung bewahrt die Zellen viel besser vor Auflösung, aber zugleich bewirkt sie, daß die Zellen weniger reaktionsfähig werden, Pilocarpin und Atropin haben eine noch schwächere gerinnungshemmende Wirkung wie Adrenalin. Verwendet man die Chloretonlösung des Adrenalins, so zeigt sich in dem entstehenden Niederschlag eine deutlich fibrilläre Anordnung, ein unsichtbares Fasernetz scheint die Körnchen des Niederschlags in Reihen anzuordnen.

Es wurden weiterhin Versuche mit 3 pCt. Formalin angestellt. Schon früher habe ich angegeben, daß durch Injektion von 10—15 ccm 3 pCt. Formalins in Limulus die Blutkoagulation sehr herabgesetzt werden kann und daß viele Zellen unter diesen Umständen ihre natürliche Gestalt bewahren, daß aber andere Zellen sich trotz dieser vorangegangenen Injektion verändern.[1]) Es wurden nun ähnliche Versuche beim Hummer und bei Platyonychus angestellt, um die Beziehungen zwischen Gerinnungshemmung und präservierender Wirkung auf die Blutkörperchen festzustellen. Es ergab sich nun, daß zuweilen nach Injektion der 3 prozent. Formalinlösung die Gerinnung bei nachheriger Entnahme des Blutes nicht ganz aufgehoben war; meist aber trat eine Verlangsamung derselben ein, oder dieselbe unterblieb zu einem großen Teile. Die Blutkörperchen waren, wie es schien, nach einer solchen Injektion zuweilen schon beim Herausfließen aus dem Tiere rund und etwas kleiner wie gewöhnlich. In einigen Fällen jedoch waren sie sofort nach dem Auffallen auf dem Objektträger noch oval. Möglicherweise trat in diesen Fällen die Formveränderung der Blutkörperchen so schnell ein, daß sie gewöhnlich auf dem Objektträger nur in runder Form gesehen werden konnten. Sehr deutlich war in allen Fällen der Umstand, daß entweder keine oder nur wenige Pseudopodien nach vorheriger Injektion von Formalin auf dem Objektträger

[1]) On the bloodlymphcells etc. loc. cit.

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für das Hummerblut charakteristische zweite Koagulation ein, jedoch gewöhnlich schwächer wie gewöhnlich.

Trat, wie das oft der Fall war, nur eine Hemmung der Koagulation ein, so sahen wir runde Blutkörperchen auf dem Objektträger; Pseudopodien wurden nicht ausgestreckt. Dann aber sahen wir infolge von Bewegung der Flüssigkeit eine Anzahl von Blutkörperchen in kleinen Häufchen zusammenkleben und zu Boden sinken. Dort bilden diese Häufchen ebenso wie die zu Boden sinkenden freien Blutkörper eine zusammenklebende Masse. Nacher mögen zuweilen auch Fasern zwischen den Zellen sichtbar werden, wobei die Fasern noch einen Kern in der Mitte enthalten konnten, ein Beweis, daß diese Fasern sich zum Teil wenigstens aus Zellen gebildet haben. Die Gerinnung kann nun auch stattfinden, wenn die Mehrzahl der Blutkörperchen keine Pseudopodien aussendet. Die Blutkörperchen können dann mit dem Beginn der Koagulation ihre Beweglichkeit verlieren, ohne daß die Zellen sich anscheinend berühren. Doch haben die meisten Zellen auch in diesem Falle Teile ihres Protoplasmas verloren, und die Möglichkeit liegt vor, daß dieses ausgetretene Protoplasma für die Verbindung der Blutzellen von Bedeutung ist. Wurde einem Hummer 3 pCt. Formalin injiziert und das während der Injektion infolge der Verletzung mit der Nadel ausströmende, mit Formalin vermischte Blut auf dem Objektträger in ebensolcher Formalinlösung aufgefangen, so bewahrten die meisten Zellen ihre ovale Form und ihre Granula. Aber selbst unter diesen Umständen waren viele Stellen vorhanden, wo schon Veränderungen in den Zellen eingetreten waren. Da, wo die Blutkörperchen am besten erhalten waren, waren alle Zellen mit Granulis versehen. Pseudopodien wurden hier nicht ausgestreckt. An anderen Stellen, wo schon Auflösungserscheinungen an den Zellen vorhanden waren, bestanden alle Übergänge von vollständig granulierten, zu granulaarmen bis zu körnerfreien Zellen. Es ist daher sehr wahrscheinlich, daß in dem unverletzten Tierkörper alle Blutzellen Granula haben, und daß nicht mit Granula versehene Blutzellen erst entstehen, wenn die Blutkörperchen außerhalb des Körpers aufgelöst werden, oder wenn z. B. durch Fremdkörper-


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wirkung im Innern des Körpers eine Läsion der Zellen stattfindet. Also den Lymphocyten (sofern diese als granulalose Blutzellen definiert werden) entsprechende Zellen kommen in aller Wahrscheinlichkeit beim Hummer nicht vor. Zugleich zeigt aber auch dieser Versuch, wie schwer es ist, ganz unversehrte Blutkörperchen außerhalb des Körpers zu fixieren, ohne zugleich einen sehr starken Niederschlag von Eiweißstoffen zu erhalten. Bei der Wirkung des 3 prozent. Formalins kombiniert sich die Wasserwirkung, die die Blutkörperchen auflöst, mit der die Zelle erhaltenden Formalinwirkung. So kann es vorkommen, daß, wo die Formalinlösung die Blutmenge überwiegt, die Wasserwirkung der Mischung stärker zur Geltung kommt, als die Formalinwirkung. Einige Beispiele mögen angeführt werden: Injiziere in einen großen Platyonychus 3½ ccm 3 pCt. Formalin. Nach etwa 30 Minuten entziehe Blut. Sobald die Blutkörperchen auf dem Objektträger gesehen werden, sind sie verändert, rund und klein, einige mit Körnern und oval, besser erhalten. Die Zellen senden nur spät und auch dann nur sehr wenige Pseudopodien aus. Das Blut koaguliert nicht. Bei Bewegung des Objektträgers agglutiniert nur eine geringe Zahl der Blutkörperchen, die übrigen Zellen bleiben meist frei beweglich. Beim Auflegen eines zweiten Objektträgers können die Zellen teilweise zu kleinen Häufchen vereinigt werden. Nach Injektion von 5—6 ccm in einen kleinen Hummer wurde ein ähnliches Resultat erhalten. Hier konnte aber festgestellt werden, daß sogleich nach dem Herausfließen die Blutkörperchen noch groß waren, daß sie dann sehr bald kleiner werden. Am nächsten Tage liegen in diesem Versuche die Blutkörperchen am Boden des Gefäßes; sie sind klein, aber wohlerhalten, und bilden zusammenklebend eine schleimartige Masse. Injiziere 15 ccm 3pCt. Formalin einem Hummer durch das Gelenk zwischen Thorax und Abdomen. Entnimm 15 Minuten später am Abdomen Blut. Die Blutkörperchen, sofort untersucht, sind meist verändert, klein, rund, mit Pseudopodien versehen. Nach einiger Zeit kleben diese Blutkörperchen zusammen und es bildet sich eine gelatinöse Masse. Blut, das nach 20 Minuten dem Rücken entnommen wurde, zeigte ebenfalls kleine Blutkörper mit Pseudopodien, doch ist die Zahl der ausgestreckten Pseudopodien geringer, als gewöhnlich.

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frei beweglich und klein gefunden; eine weitere Veränderung trat nicht ein und sie klebten nicht zusammen.

Diese Beispiele zeigen, welche Variationen hier gefunden werden; immer treten eine Hemmung der Gerinnung und eine Einschränkung der Blutkörperchenveränderungen ein. Wenn eine zweite, gelatinöse Gerinnung nach Injektion von Formalin eintrat, unterblieb dennoch die sekundäre, früher beschriebene hyaline Ausbreitung der Blutkörperchen. Auch in Formalin gingen einzelne Blutkörperchen zu Grunde. Durch Auflegen eines zweiten Objektträgers konnte gezeigt werden, daß sogar unter Formalinwirkung die einzelnen Blutkörperchen ihre Dehnbarkeit jedenfalls noch im Anfang beibehielten.

Einfluß der Wärme: Erwärmen der Tiere vor dem Entziehen des Blutes hat einen ähnlichen hemmenden Einfluß auf die Gerinnung und die Gestaltsveränderungen der Blutkörper wie Mischung des Blutes mit Formalin. Dabei ist die Wärmezufuhr, die nötig ist, um dieses Resultat hervorzubringen, verschieden bei den verschiedenen Arten, die untersucht werden. Und zwar fand sich eine gewisse Beziehung zwischen der Temperatur, bei der die Tiere starben und der Temperatur, bei der das Blut seine Koagulierbarkeit verlor.

Kleine Limuli, die allein zu diesen Versuchen verwandt wurden, starben, wenn sie in Seewasser gebracht wurden, das allmählich erhitzt wurde, bei einer Temperatur von 52 bis 54° C. Bei 37—38° fangen die Tiere an sehr lebhafte Bewegungen auszuführen. Sie sterben unter starker Beugekontraktion der Extremitäten. Hält man diese Tiere konstant bei 40°, so sind dieselben noch nach einer Stunde lebendig und werden in gewöhnlichem Seewasser sehr schnell wieder normal. Bei 45° gehalten, starben sie nach Ablauf von 30 Minuten.

Libinia wird ebenfalls bei schnell steigender Temperatur sehr lebhaft, sobald 37—38° erreicht ist und stirbt bei 43°; ein etwas größeres Tier starb erst einige Minuten später, als die Temperatur des Wassers 49° erreicht hatte; auch hier tritt der Tod unter Beugekontraktion ein. Bei 40° gehalten, stirbt eine Libinia nach 10 Minuten, bei 45° gehalten nach 6 Minuten.



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Hummer werden ebenfalls sehr lebhaft beweglich bei 37—38° und sterben zwischen 40° und 43° ebenfalls in Beugekontraktion.

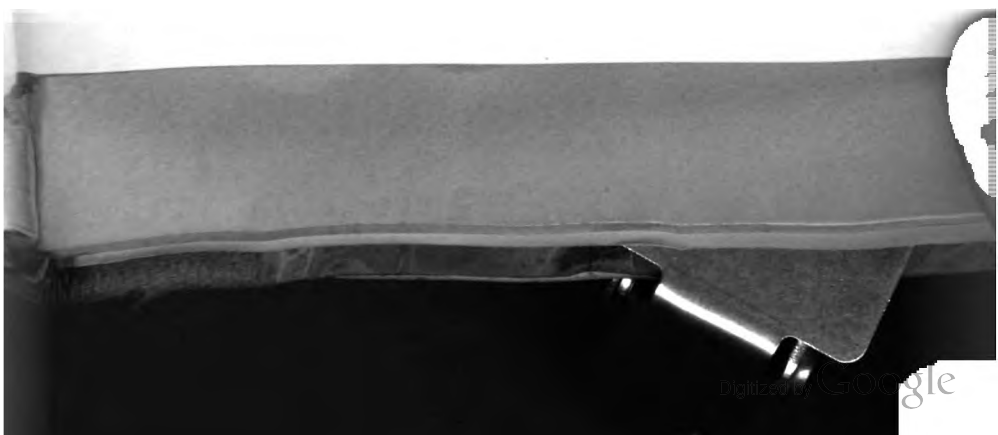
Dementsprechend verliert das Blut eines jungen Limulus seine Koagulationsfähigkeit erst, wenn das Tier 30—40 Minuten bei 52—54° gehalten worden war, während das Blut anderer Tiere schon sehr stark an Koagulationsfähigkeit einbüßt, wenn die Tiere vor der Entnahme des Blutes eine halbe Stunde bei 46° gehalten werden.

So wurde z. B. bei einem Hummer die Blutgerinnung aufgehoben, nachdem das Tier 40 Minuten auf 48—49°, bei einem anderen, nachdem es 30 Minuten auf 45—46° erwärmt war. Erst nach einer Stunde trat am Boden des Gefäßes in diesem Falle etwas Gerinnung ein. Bei einer Libinia, die auf 43° erwärmt war, bildeten sich nur kleine Häufchen zusammenklebender Zellen. Bei einem Platyonychus wurde durch 30 Minuten langes Erwärmen auf 45—46° die Koagulation nur etwas verzögert. Bei einem kleinen Limulus, der 10 Minuten auf 52°, 30 Minuten auf 54° erhitzt war, wurden nur einige kleine Häufchen von Zellen gebildet, die meisten Blutkörperchen blieben frei. In einem anderen, der ¾ Stunden auf 52° erwärmt war, trat keine Koagulation ein. Kein Fett trat mit dem Blut heraus. Bei Bewegung des Objektträgers agglutinierten später einige Blutkörperchen. In einem Limulus hingegen, der nur einige Minuten auf 54° erwärmt war, gerann das Blut. Verringert man die Zeit, während welcher die Tiere dieser Temperatur ausgesetzt werden, so wird die Gerinnung nur ein wenig gehemmt, ohne sehr stark beeinflußt zu werden. Dasselbe findet auch statt, wenn eine noch höhere Temperatur nur während einiger Minuten erreicht wird. Wir finden also alle Abstufungen von Gerinnungshemmung bis zu völliger Aufhebung der Gerinnung, gerade so wie das beim Formalin der Fall war. Bei bloßer Hemmung kann man z. B. beobachten, daß die Zellen nach dem Verlassen des Körpers erst ganz frei auf dem Objektträger beweglich bleiben; daß jedoch kurze Zeit später, sowie eine Bewegung des Objektträgers stattfindet, benachbarte Blutkörperchen zu kleinen Häufchen sich vereinigen, „agglutinieren“; die relative Menge der agglutinierenden und der freibleibenden Blutzellen wechselt in den einzelnen Fällen. Die Zellhäufchen und

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dem Objektträger oder in dem Gefäße das Blut gerinnt, obwohl man unter dem Mikroskope nur Häufchen von Zellen sieht, die in der Flüssigkeit frei beweglich sein können. Nach einiger Zeit mögen auch zwischen diesen Zellmassen Fasern sichtbar werden. Wie bei vorheriger Injektion mit Formalin erscheinen auch nach vorherigem Erwärmen des Tieres oft die Blutkörperchen beim Verlassen des Tieres rund; die Anzahl der erhaltenen Granula kann hierbei wechseln. Zuweilen war jedoch die ovale Form noch sichtbar, als die Zellen auf dem Objektträger untersucht wurden; die Zellen veränderten sich aber in solchen Fällen sehr schnell. Natürlich besteht die Möglichkeit, daß die Zellen sich auch nach Wärmewirkung erst während des Verlassens des Tierkörpers ändern; doch ist das unwahrscheinlich. Nach stärkerem Erwärmen strecken die Blutkörperchen auf dem Objektträger keine Pseudopodien mehr aus, in anderen Fällen strecken sie erst später wie gewöhnlich und dann eine geringere Anzahl von Pseudopodien aus. Mit oder ohne Pseudopodien können diese Blutkörperchen zu Häufchen zusammenkleben, und es kann hierbei der Anschein der Bildung einer Gallerte entstehen. Die Bildung einer Gallerte kann stattfinden, ohne daß sich die Blutkörperchen berühren; doch kann man wenigstens zuweilen auch hier sehen, daß Plasmoschise stattgefunden. Es mag also ausgetretenes Protoplasma die Zellen verbinden. Die am tiefsten liegenden Zellen können auch unter diesen Umständen am Objektträger festkleben.

Nach kurzem Erhitzen auf 59° und darauffolgendem 10 Minuten dauerndem Erwärmen auf 52° kann eine Gerinnung des Blutes unter Entstehen von Zellhäufchen stattfinden, dann aber wurde bei längerer Beobachtung festgestellt, daß die unter gewöhnlichen Umständen während der nächsten zwei Stunden stattfindende hyaline Ausbreitung der Zellen unterblieb. Die Zellen befanden sich in einem Zustand der „Wärmestarre". Doch konnte die hyaline Ausbreitung erfolgen, wenn die Temperatur nicht so stark erhöht worden war. Es fehlt auch wärmestarren Blutkörperchen die Reaktionsfähigkeit nicht ganz. Bei Druck mit einem aufgelegten Objektträger können auch hier Zellen



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„explodieren", wonach ausgeflossene Granula sichtbar werden. Tropfen von hyalinem Protoplasma mögen sich auch hier der Peripherie anlegen. Solche Tropfen können frei werden, in der Flüssigkeit umherschwimmen und nachher an Zellhaufen festkleben. Ähnliches wurde auch in dem Formalinblut beobachtet. Auch können Zellen hier, ohne daß ein Druck ausgeübt wurde, ganz oder teilweise zerfließen. Die Granula an der Peripherie der Zelle unter der transparenten Grenzschicht können so groß werden, daß man im Zweifel ist, ob man es hier mit den gewöhnlichen Körnern zu tun hat, oder ob eine Umwandlung derselben in kleine Tropfen stattgefunden hat. Auch können einzelne Zellen noch nach stattgefundenem Erwärmen in Fasern ausgezogen werden. Doch finden alle diese Umwandlungen nicht sehr häufig statt. In einzelnen Fällen konnte beobachtet werden, daß die Zellen schon zu Häufchen vereinigt das Tier verließen. In einem solchen Falle konnte die Ursache hierfür erkannt werden. Beim Eröffnen des Tieres zur Entnahme des Blutes geriet Rost der Scheere in das Tier. Um diese kleinen Rostpartikelchen als Centrum bildeten sich die Zellhaufen. Also ein mechanischer Reiz war die Ursache der Bildung von Zellhaufen. Es wurde auch versucht, ob eine Temperaturerhöhung, die nicht im stande war, die Gerinnung zu verhindern, wenigstens auf den zeitlichen Ablauf des Gerinnungsvorgangs einwirke. Es fand sich, daß ein Erwärmen eines Limulus auf 47° mit sofortigem nachherigem Abkühlen eine geringe Verzögerung des Gerinnungsvorganges bewirkt. Ebenso trat diese Verzögerung beim Erwärmen eines Limulus auf 48°—50° ein, in diesem Versuch wurde nachher das Blut in Seewasser, das bei der gleichen Temperatur (48—50°) gehalten wurde, aufgefangen. Es wurde hierbei die Zeit bestimmt, die nach dem Ausfließen des Blutes verfloß, bis eine Fadenbildung auf dem Objektträger bemerkbar wurde. Falls nämlich das Blut beim Auffangen auf dem Objektträger in Seewasser hin und her bewegt wird, verwandelt sich das Blut nicht in eine Gallerte, sondern in einzelne Fäden und zwischen den Fäden befindet sich frei bewegliche Flüssigkeit. Gewöhnlich ist diese Fadenbildung 19 Sekunden nach dem Auffallen des Blutes auf den Objektträger deutlich. Bei der angegebenen mäßigen Erwärmung trat die Fadenbildung erst nach 24—30 Sekunden ein. Der Unterschied ist also nicht sehr bedeutend.

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Gerinnung hemmenden Temperaturen ausgesetzt wurden, ni
nicht selten aus der Wunde mit dem Blute noch andere Kö
bestandteile aus, wie Fetttropfen, Bindegewebszellen, Mu
stückchen oder Leberpankreassaft. Letzterer floß bei Li
und Platyonychus ocellatus sogar zuweilen dann aus, wenn
Blut den Extremitäten entnommen wurde. Das Erwärmen
also hier offenbar einen macerierenden Einfluß auf das
doch konnte oft die Gerinnung stark beeinflußt werden,
daß anscheinend andere Bestandteile dem Blut beigemischt w
Diese Beimischung war nicht der wesentliche Faktor bei
durch die Wärmung bewirkten Gerinnungshemmung. Hing
war es deutlich, daß bei Libinia, Platyonychus und Hum
die bei niedrigerer Temperatur starben wie die jungen Li
auch das Austreten verschiedener Gewebsbestandsteile aus
Tiere bei niedrigerer Temperatur stattfindet, als bei Lim
Diese Vermischung von Körperbestandteilen mit dem Blut
vielleicht für den Tod des Tieres von Bedeutung sein; viell
besteht aber auch nur ein indirekter Zusammenhang, inden
den erstgenannten Tieren alle oder viele Körperzellen, i
sondere die Nervenzellen, bei niedrigerer Temperatur verä
und getödtet werden, wie bei dem jungen Limulus. A
Zellen und Fett waren auch kleine Häufchen granulärer M
wahrscheinlich geronnenen Eiweißes, zuweilen im Blut vorha
ein Teil dieser granulären Masse mag aus zerfallenen Blutz
entstanden sein.

Es wurde festzustellen versucht, ob Einwirkung von
die Gerinnung verhindern kann. Ein erwachsener Lin
wurde 7 Stunden auf Eis gelegt, dann das Blut sofort in
0° gehaltenen Schalen aufgefangen. Das Blut begann
dem Verlassen des Körpers wie üblich zu gerinnen.
zuletzt herausfließende Teil des Blutes gerann in Strä
und nicht als gelatinöse Masse, wie das für die die Hauptn
derselben der Fall ist. Es ergab sich aber, daß dasselbe
bei gewöhnlichem Limulusblut geschieht, in anderen Vers
wurden junge Limuli auf Eis gehalten und ihr Blut in Eisw
aufgefangen; falls eine Gerinnungsverzögerung stattfand, w
ich mich nicht sicher überzeugen konnte, betrug dieselbe j



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falls nicht mehr als einige Sekunden. Diese Versuche wurden nur am Limulus angestellt. In einem anderen Versuche wurden ein erwachsener Limulus und drei kleine Limuli 2½ Stunden in eine Eis und Salzmischung gebracht. Beim Herausnehmen waren sie gefroren und in Beugekontraktion. Die Blutkörperchen wurden in diesem Versuch in den ausgeschnittenen Kiemen, also noch innerhalb des Tieres unter dem Mikroskop beobachtet und es zeigte sich, daß die Blutkörper schon vor dem Verlassen des Körpers rund waren; in manchen Zellen waren die Granula erhalten, andere Zellen hatten dieselben teilweise oder ganz verloren. In dem Tier selbst war das Blut nicht geronnen. Doch gerann es nach dem Herausfließen, nachdem die Tiere vorher einige Zeit in gewöhnlichem Seewasser gelegen hatten, in der gewöhnlichen Weise. Hier streckten denn auch die Blutzellen auf dem Objektträger Pseudopodien aus. Auf die genaueren zeitlichen Verhältnisse der Gerinnung war bei diesem Versuch nicht geachtet worden. Bei anderen Arthropoden fanden Loewit und Halliburton eine beträchtliche Gerinnungshemmung bei Erniedrigung der Temperatur.

Über die Einwirkung des Leberpankreassaftes auf die Blutgerinnung und die Blutkörperchen.

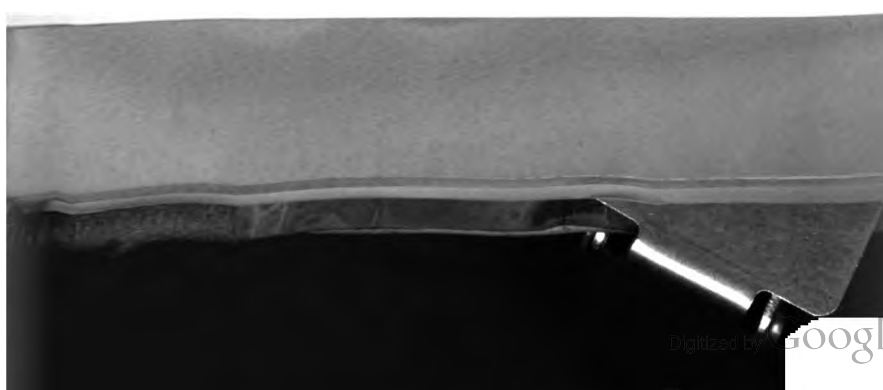
Fangen wir das Blut eines der zu diesen Untersuchungen benutzten Tiere in dem in den Verdauungskanal der Arthropoden sezernierten Pankreaslebersaft auf, so sehen wir eine deutlich hemmende Einwirkung auf den Gerinnungsvorgang. Die in diesen Versuchen verwandte Flüssigkeit war gewöhnlich ein Gemisch des Leberpankreassekrets mit einer kleinen Menge Blut und Verdauungsprodukten. Häufig befanden sich auch Bakterien in dieser Flüssigkeit, doch der wirksame Bestandteil war wahrscheinlich Leberpankreassaft. Das Sekret aller untersuchten Arthropoden war wirksam, doch war der Hummer am geeignetsten, um einen klaren Pankreassaft zu erhalten.

Gewöhnlich bewirkten die Mischung des Blutes und der verschiedenen Verdauungsflüssigkeit Niederschläge, die aber nicht immer gleich stark waren. Hummerverdauungssaft gab gewöhnlich mit Limulus- und Libiniablut einen starken Niederschlag. Zuweilen konnte auch der Niederschlag ganz fehlen oder gering

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ein ziemlich beträchtliches Präcipitat, keinen Niederschlag hingegen mit Hummerblut. Doch rühren diese Niederschläge wohl von dem dem Pankreassekret beigemischten Blute her. Blutserum von Limulus gibt nämlich ein Präcipitat, wenn es mit Libiniablut, und weniger schnell, wenn es mit Hummerblut gemischt wird. Hummerblut gibt gewöhnlich keinen Niederschlag mit Libinia- oder Platyonychusblut. Mit seinem eigenen Verdauungssaft gibt das Blut eines Tieres gewöhnlich keinen Niederschlag. Fangen wir nun in Libiniaverdauungssaft Limulusblut in einer Schale auf, so tritt, wenn die Menge des Verdauungssaftes überwiegt, nicht wie gewöhnlich Bildung einer schleimigen oder spinnwebenartigen Substanz ein, indem die Blutkörperchen sich nicht zu einer zusammenhängenden Masse vereinigen, sondern statt dessen sehen wir, wie sich allmählich kleine Flöckchen bilden, die aus Zellen und einem Präcipitat bestehen. Die Präcipitatbildung ist hierbei aber unwesentlich, da die Wirkung des Pankreassaftes auch stattfindet, ohne daß ein Niederschlag sich bildet. Auch auf dem Objektträger entstehen unter solchen Umständen an Stelle des zusammenhängenden Coagulums kleine isolierte Häufchen von Zellen. Fäden, die solche Häufchen verbinden, können teilweise wieder aufgelöst werden.

Unter dem Mikroskop beobachtet man nun, daß viele Zellen in dem im Pankreassaft aufgefangenen Limulusblut frei bleiben, andere aber kleine Häufchen bilden, die im Anfang frei umherschwimmen, dann aber oft zu Boden sinken. Am Boden mögen sie zuweilen festkleben, gewöhlich aber bleiben sie frei beweglich. Die Zellen strecken auch hier gewöhnlich im Anfang, auch wenn sie ganz frei in der Flüssigkeit umhergetrieben werden, Pseudopodien aus. Häufig aber schwinden diese Pseudopodien nach einiger Zeit, sie werden verdaut. An Stelle eines spitzen Fortsatzes mag dann zuerst ein stumpfer Ausläufer erscheinen. Zuweilen nun konnte ich beobachten, daß diese mit Pseudopodien versehenen, umhergetriebenen Zellen beim Zusammenstoßen mit ihren Pseudopodien aneinanderkleben und so sich zu Häufchen vereinigten. Wenn diese Häufchen oder Zellen zu Boden fielen, so klebten sie entweder garnicht fest oder sandten eine geringe Zahl von Protoplasmafortsätzen aus, mit denen sie am Objekt-



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träger für einige Zeit befestigt blieben. Falls ein Niederschlag entstand, so trat dieser zuerst ganz diffus in Form feiner Körnchen auf, derselbe zog sich aber dann wie durch unsichtbare Fasern gezwungen auf die Verbindungslinie der Häufchen zusammen. War der Niederschlag sehr stark, so konnte er verhindern, daß die Zellhäufchen am Boden klebten. Falls nun ein größerer Tropfen Blut auf den Objektträger auffiel, so konnten die centralgelegenen Teile des Blutes gerinnen. Die am Boden liegenden Zellen mochten dann den Beginn der beschriebenen hyalinen Ausbreitung zeigen; gewöhnlich aber blieb diese unter der Wirkung des Pankreassekretes nur unvollständig. Die gelatinöse Gerinnung des Hummerblutes unterblieb unter diesen Umständen. 10—30 Minuten nach Ausfließen des Blutes konnten nun an den Blutkörperchen sehr starke Auflösungserscheinungen beobachtet werden. Manche Blutkörper schwollen an; sie bildeten eine Kugel, die durchlöchert war, bald blieben nur einige Reifen übrig. Auch große Vakuolen konnten in den Zellen entstehen. In dem erwärmten Blute waren zuweilen ebenfalls geschwollene Zellen vorhanden, doch war hier nicht mit Sicherheit auszuschließen, daß eine Beimengung fremdartiger Zellen vorlag, die durch die Maceration der Gewebe ins Blut gelangten. Diese durchlöcherten Zellen wurden nun schnell weiter zerstört; sie zerfielen in verschieden große Körner, die gewöhnlich eine sehr starke Molekularbewegung ausführten. So konnte auch nachträglich scheinbar ein granulöses Präcipitat entstehen. Die Geschwindigkeit und Vollständigkeit, mit der diese Auflösung stattfand, war verschieden. Zuweilen war schon nach 30 Minuten kein Blutkörperchen mehr zu sehen. Auch freischwimmende Kerne waren vorhanden. Zuweilen wurde gleich im Anfang die Ausstreckung von Pseudopodien verhindert, die runden Zellen kleben dann zum Teil aneinander. Wir sehen also, daß der Leberpankreassaft eine stark verdauende Wirkung auf die Blutzellen hat. Vielen Bakterien, sowie gewissen Protozoen gegenüber ist er ganz wirkungslos (ob in Bezug hierauf zwischen den verschiedenen Protozoen Unterschiede bestehen, soll noch untersucht werden). Der Darmsaft verliert seine Wirksamkeit auf das Blut nach Erwärmen zu 65—70° während einer halben Stunde; nicht aber bei Erwärmen auf 56°. Daß bei Mischung

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Im übrigen hat aber das Serum eines Tieres, wenn das Blut eines anderen Tieres in ihm aufgefangen wird, keinen Einfluß auf die Gerinnung dieses Blutes. Sie findet gewöhnlich gerade so wie in Seewasser statt. Möglicherweise besteht ein Unterschied darin, daß in Limulusserum die Blutkörperchen weniger zahlreiche und weniger spitze Pseudopodien aussenden, wie in Libinia oder in Hummerserum. Doch müssen darüber weitere Beobachtungen angestellt werden.

In einigen Fällen wurde das Blut in dem Blutserum von Schildkröten (Chelopus guttatus und Chrysemis picta) aufgefangen. Die Blutkörperchen schwollen hier sehr an und klebten zusammen. Die Quellung des Zellprotoplasmas war oft so stark, daß die Kerne allein sichtbar blieben. In 0,7 pCt. Salzlösung fand auch eine Quellung der Zellen statt. Jedoch bestand ein bemerkenswerter Unterschied darin, daß in der isotonischen Salzlösung die Zellen Fortsätze ausstreckten, und die hyaline Ausbreitung stattfand; daß aber in dem Schildkrötenserum dieses unterblieb. Es müssen in dem Serum Substanzen vorhanden sein, die diese Ausbreitung verhindern.

Die in Schildkrötenserum zusammenklebenden Zellen des Limulus-, Libinia- oder Hummerblutes können durch ihr Aufschwellen und Zusammenkleben weiche Flocken bilden. Doch findet man hier zuweilen auch zu Fasern ausgezogene Zellen, sowie lange Fasern, deren Ursprung nicht zu erkennen ist. Das Schildkrötenserum hindert also nicht die Koagulation. Einzelne Blutzellen behalten einen Teil ihrer Granula, und nachdem der größte Teil der Zelle aufgelöst ist, kann man dann noch Kerne mit einigen Granula als Reste einer Zelle finden.

Es wurde weiterhin versucht, ob durch Injektion von Limulusblutserum in Schildkröten Substanzen produziert werden könnten, die die Koagulation von Limulusblut oder die Blutkörperchen von Limulus spezifisch beeinflussen. In eine Anzahl von gewöhnlichen Schildkröten (Chrysemis picta, Chelopus guttatus) und eine Chelydra serpentina wurden 12—16 mal jeden 2. bis 3. Tag (in kleinen Schildkröten) je $\frac{1}{2}$ ccm, in Chelydra 1 oder 2 ccm durch Filtrirpapier filtriertes Limulusserum injiziert. Sechs bis acht Tage nach der letzten Injektion wurde das Blut ent-


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nommen und das Serum des defibrinierten und dann centrifugierten Blutes auf seine Wirksamkeit geprüft. Wenn Limulusblut auf dem Objektträger in solchem Serum aufgefangen wurde, so fand die Koagulation in gleicher Weise statt, wie in dem in Kontrollserum von gewöhnlichen Schildkröten aufgefangenen Limulusblut. Auch die Einwirkung auf die Blutzellen von Limulus war dieselbe. Mischte man das Serum von solchen vorbehandelten Schildkröten mit der gleichen Menge Limulusblutserum, so bildete sich kein wesentlich stärkeres Präzipitat, als wenn Limulusserum mit 0,6 pCt. Natriumchlorid-Lösung oder mit gewöhnlichem Schildkrötenserum gemischt wurde. Kleine Flocken bilden sich in Limulusblutserum allmählich spontan. Vielleicht war die Zahl der Flocken nach einer halben bis einer Stunde in dem mit dem Serum vorbehandelter Schildkröten gemischten Limulusserum etwas größer als in dem Kontrollblut, aber der Unterschied war in wiederholten Versuchen zu gering, als daß er als spezifisch angesehen werden konnte. In weiteren Versuchen wurde Serum vorbehandelter Schildkröten in den Kopf kleiner Limuli injiziert, um eine etwaige spezifische Wirkung innerhalb des Tierkörpers zu untersuchen.

So wurden in einem Versuch am 22. September 1902 je $\frac{1}{4}$—$\frac{1}{3}$ ccm Serum einer vorbehandelten Riesenschildkröte in den Kopf von 18 kleinen Limuli injiziert. 9 Kontrolltieren wurde die gleiche Menge von gewöhnlichem Schildkrötenserum eingespritzt. Alle diese Limuli blieben normal und noch nach 8 Tagen schwammen sie lebhaft im Seewasser. In einem zweiten Versuch wurden einem Zwerglimulus 1 ccm, einem zweiten $\frac{1}{4}$ ccm Blutserum von vorbehandelten Schildkröten ebenfalls in den Kopf injiziert. In diesem Falle starben die Tiere nach 2 Tagen, während die Kontrolltiere, welche mit normalem Schildkrötenserum injiziert worden waren, am Leben blieben. Das Blut der ersten beiden Tiere zeigte den Charakter des Blutes sterbender Tiere, es war sehr arm an Blutkörperchen und produzierte nur wenig Koagulum. Es ist wahrscheinlich, daß die Tiere hier infolge von Blutverlust starben. Die Einstichöffnung war in diesem Falle nicht sorgfältig durch Collodium verschlossen worden, und obwohl das Blut vom Limulus schnell koaguliert, ist es sehr wenig geeignet auch nur die kleinsten Ein-

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stichöffnungen so weit zu verschließen, daß das Tier vor allmählichem Verblutungstod bewahrt bleibt. (Schon im Sommer 1900 begann ich einzelne Versuche der Immunisation wirbelloser Tiere. Im Sommer 1901 versuchte ich gemeinschaftlich mit H. G. Wells Kaninchen gegen Spermatozoen und Eier von Arbacia zu immunisieren. Eine Notiz über diese Versuche findet sich in den Transactions of the Chicago Pathological Society, Vol. V, No. 3 (Dezember 1901). Unsere Ergebnisse waren im allgemeinen die gleichen wie die der wohl etwas früher angestellten, aber nach Beendigung unserer Versuche publizierten Untersuchungen von Dungerns (Zeitschrift für allgemeine Physiologie, Bd. I, 1). Wir fanden jedoch, daß das Serum von mit Eiern und Spermatozoen von Arbaciaimmunisierten Kaninchen nicht nur auf die Spermatozoen anderer Echinodermen, sondern auch auf Spermatozoen von Würmern, die untersucht wurden, stark agglutinierend wirkte. Letzten Sommer machte ich neben den oben beschriebenen weitere Versuche, die unter anderem bezweckten, in Limulus Koaguline gegen Milch hervorzubringen. Ich injizierte in mehrere Limuli in Abständen von 5—6 Tagen zweimal in einer Versuchsreihe, je 10 ccm, in einer anderen je 15 ccm Kuhmilch. Acht Tage nach der letzten Injektion wurde das Serum eines solchen Limulus in verschiedenen Proportionen mit unverdünnter und mit durch Seewasser verdünnter Kuhmilch gemischt. Es trat keine spezifische Reaktion ein. Doch war das Blut dieser Tiere nicht normal, sondern hatte den Charakter des Blutes eines anämischen Tieres. Wahrscheinlich hatte das Tier nach der Injektion Blut verloren. Begonnene Versuche mit Injektion von Diphtherietoxin konnten nicht zu Ende geführt werden.)

Eine deutlich hemmende Wirkung auf die Gerinnung des Blutes von Limulus hatten in einer Reihe von Versuchen Gelatinelösungen. Es wurden Lösungen verschiedener Koncentration mit destilliertem Wasser benutzt, sowohl ganz dünnflüssige, wie stark syrupöse. In allen diesen findet Gerinnungshemmung oder völlige Aufhebung der Gerinnung statt, wenn in der gewöhnlichen Weise das schnell aus dem Tier strömende Blut in einer Schale in einem Überschuß von Gelatine aufgefangen wird. An einzelnen Stellen finden wir auch hier kleine

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Häufchen von zusammenklebenden Zellen, die aber nicht von einer extracellulären gallertigen Fibrinmasse zusammengehalten werden, da sie leicht zu trennen sind. An vielen Stellen sind nur isolierte Zellen vorhanden. Zuweilen finden wir Fäden; der Rest eines Kernes oder einige Granula im Innern dieser Fasern zeigten an, daß eine solche Faser aus einer ausgezogenen Zelle entstanden war. Wurde ganz dünne Gelatine benutzt, so mußte man sehr vorsichtig sein, eine starke Erschütterung der Gelatine-Blutmischung zu vermeiden, da sonst Gerinnung eintreten konnte. Man mußte es z. B. vermeiden, die Blutgelatinemischung aus der Schale auf den Objektträger in einer engen Pipette zu übertragen; da hierbei die beim Ein- und Ausfließen entstehende starke Reibung oft genügte, um Agglutination der Zellen und Faserbildung zu bewirken. Mit weiten Pipetten kann man hingegen bei vorsichtiger Behandlung auch das in ganz dünnen Gelatinelösungen aufgefangene Blut, ohne Gerinnung hervorzurufen, auf den Objektträger übertragen.

In dickeren Lösungen sieht man viele freie Granula. Zum Teile rühren diese von zu Grunde gehenden Zellen her. Im Anfang, als mir noch keine reine Gelatine zur Verfügung stand, benutzte ich Lösungen eines kommerziellen Leimes (Lepages glue). Derselbe enthält anscheinend Terpentin. Dieser hatte dieselbe gerinnungshemmende Wirkung, wie die reine Gelatine. Nur schwollen hierbei bei Zusatz von destilliertem Wasser die Zellen stärker auf, wie in der reinen Gelatine; diese geschwollenen Zellen hatten oft eine epithelähnliche Form. Sie legten sich auch zuweilen in Reihen aneinander. Hier sehen wir nun Granula verschiedener Größe in den Zellen. Zuweilen liegen sie direkt unter der transparenten, membranähnlichen Außenschicht. Zuweilen scheinen sie zu Stäben und zu einer die Peripherie einer halben Zelle einnehmenden relativ breiten, halbflüssigen Schicht zusammenzufließen. Viele Zellen schmelzen halb oder ganz ein. In einzelnen Zellen bleiben nur die peripherischen Tropfen, bezw. Granula konzentrisch um den in der Mitte gelegenen Kern angeordnet erhalten. In der unreinen Gelatinelösung waren Niederschläge vorhanden, die allmählich stärker wurden; diese waren zum Teil wohl auch durch Zusammenbrechen der Zellen bedingt. Wurde die Gelatine in Seewasser anstatt in destilliertem Wasser gelöst, so blieben die Zellen kleiner.

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Über die Wirkung des Harnstoffs und des Peptons auf die Gerinnung.

Fängt man Limulusblut in konzentrierten, jedoch nicht gesättigten Lösungen von Harnstoff auf, so tritt Gerinnung ein. In 38 pCt. Lösung von Harnstoff in destilliertem Wasser und in einer völlig gesättigten Harnstofflösung wird auch die Gerinnung des Hummerblutes nicht verhindert. Es ist bemerkenswert, daß unter diesen Umständen das Gerinnsel des Hummerblutes, das sich sonst stark kontrahiert, sodaß es dem Säugetierfibrin gleicht, hierbei ganz gallertig bleiben kann und so dem zweiten Coagulum des Hummerblutes ähnlich wird. Bringen wir das gewöhnliche, der ersten Gerinnung entstammende, fädige Hummerfibrin aus Wasser, in dem es einen opaken und brüchigen Charakter annimmt, in eine gesättigte oder halbgesättigte Harnstofflösung, so wird dasselbe schon nach wenigen Minuten aufgehellt, schwillt auf und wird weniger brüchig. Zum Vergleich in Glycerin gelegtes Gerinnsel wird transparenter wie das im Wasser liegende, schwillt aber nicht auf. Untersuchen wir mikroskopisch die in diesen Lösungen vor sich gehenden Veränderungen der Blutzellen des Limulus und des Hummers, so finden wir ein bedeutendes Aufschwellen und Hyalinwerden der Zellen. Die Zelle kann ganz unsichtbar werden, sodaß nur noch der Kern hervortritt. In anderen Fällen bleibt nur noch eine Art Membran übrig, welche die Peripherie der Zelle andeutet; dieser Membran liegt der Kern an. Die Granula schwinden häufig ganz; zuweilen aber bleiben große Granula (Tropfen) in der Peripherie der Zelle sichtbar. Weiterhin brechen viele Zellen ganz zusammen, und ihr Zellprotoplasma wird zu Fäden ausgezogen; in diesen Fäden können noch Körner liegen. Läßt man einen zweiten Objektträger auf den ersten auffallen, so bildet sich infolge des mechanischen Eingriffs etwas fädiges Fibrin. An anderen Stellen sieht man dann noch fetzenartige Reste von Zellen. Infolge mechanischer Bewegung werden allmählich mehr Fasern gebildet. Das so entstehende Fibrin ist sehr weich, weicher als dasjenige, welches sich in anderen Lösungen, z. B. in konzentrierten Lösungen von Kaliumchlorid oder in Hydrochinonlösungen bildet.

Harnstoff wirkt also in ähnlicher Weise auf das Zellproto-

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plasma und auf das entstehende Fibrin. Es ist nun von Interesse, daß starke Lösungen von Albumosen (Wittes Pepton) einen ganz ähnlichen Einfluß auf den Charakter des Fibrins haben, das sich beim Auffangen des Hummerblutes in diesen Lösungen bildet, wie Harnstoff. Verwendet wurde sowohl eine 25 prozent. Lösung, als auch etwas schwächere Lösungen. Das Fibrin, welches sich aus dem in Pepton aufgefangenen Blute bildet, bleibt gallertig und kontrahiert sich nicht oder nur sehr wenig. Noch am nächsten Morgen ist dieses Gerinnsel ganz weich, und gelatinös; mikroskopisch erkennt man zu dieser Zeit in dem Coagulum nur mehr Kerne und einzelne Granula.

Legt man jedoch bereits kontrahiertes Fibringerinnsel in eine Peptonlösung, so findet nicht die für in Harnstoff gelegtes Fibrin charakteristische Veränderung statt. Solches Fibrin ist am nächsten Tag durch die Einwirkung des Peptons sehr dunkel geworden. Auch die Peptonlösung selbst wird dunkler. Die Versuche über die Wirkung des Peptons wurden bisher nur am Hummer angestellt; weitere Versuche müssen zeigen, ob auch Limulusblut beim Auffangen in stärkere Peptonlösungen gerinnt.

Limulusblut, welches in Glycerin oder in Chloroform aufgefangen wird, gerinnt. Die Zellen werden in diesen Flüssigkeiten nicht präserviert. Wird soviel Pepton, als genügen würde, um die Blutgerinnung bei einem kleinen Hunde aufzuheben, in einen Limulus injiziert, so wird dadurch die Gerinnung des ausfließenden Limulusblutes nicht gehemmt. Auch nach Injektion von 40 ccm Glycerin in das Tier in das Rückenmark, gerinnt das Blut nach dem Herausfließen wie gewöhnlich. In einigen Versuchen wurde geprüft, ob es möglich ist, in der von Haycraft bei anderen Wirbellosen versuchten Weise das Blut von Limulus dadurch flüssig zu erhalten, daß eine vollständig mit Öl benetzte Pipette in das Tier eingeführt und das Blut so direkt in Öl aufgefangen wurde, ohne Berührung mit den Geweben oder der Luft; die Gerinnung konnte in dieser Weise nicht verhindert werden. Auch wenn das Blut direkt unter Öl aufgefangen wurde, indem man das Tier unter Öl tauchte, bevor das Blut entzogen wurde, konnte die Gerinnung nicht verhindert werden.

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Wir haben schon im vorstehenden wiederholt erwähnt, daß mechanische Einflüsse die Zellen verändern. Diese Veränderungen wurden auf dreierlei Weise untersucht. Ganz leichte Eiwirkungen wurden schon dadurch erzielt, daß man auf einen Objektträger in einer Flüssigkeit einen Tropfen Blut auffing und dann die Flüssigkeit durch Neigen des Objektträgers nach einer bestimmten Richtung bewegte. Stärkere Wirkungen konnten dadurch erreicht werden, daß mit feinen Nadeln ein Zug in bestimmter Richtung auf einzelne Zellhaufen ausgeübt wurde oder daß Zellen mit einer Nadel auf dem Objektträger geschlagen wurden. Druckwirkungen wurden dadurch erzielt, daß ein zweiter Objektträger auf das auf einen Objektträger befindliche Blut aufgelegt und dann ein schwächerer oder stärkerer Druck ausgeübt wurde. Wenn der obere Objektträger von dem unteren abgehoben wurde, wurde auf die vorher gedrückten Zellen ein Zug ausgeübt. Das Hauptergebnis aller dieser Versuche läßt sich dahin zusammenfassen, daß unter dem Einfluß von auf diese Zellen ausgeübten Druck- und Zugwirkungen der zellige Charakter schwindet und eine Masse entsteht, die sich von dem gelatinösen oder fibrillären Fibrin nicht unterscheiden läßt, die makroskopisch wie Fibrin aussieht, die ähnliche physikalische Eigenschaften hat, indem sie nämlich, wie das besonders unter dem Mikroskop zu erkennen ist, klebrig, dehnbar und elastisch ist. Das Fasersystem in das die Zellen umgewandelt werden können, ist mikroskopisch nicht von dem aus dem Blutplasma entstehenden Fibrin zu unterscheiden. Es läßt sich ferner nachweisen, daß das Protoplasma der Blutzellen ursprünglich keine fibrilläre Struktur hat, daß aber durch Zugwirkungen sekundär ein fibrillärer Charakter der Zellen zustande kommt; daß diese Fibrillen sowohl das Protoplasma einer Zelle in Mehrzahl durchziehen können, wobei der zellige Charakter noch erhalten bleibt, oder auch daß ganze Zellen zu Fasern umgewandelt werden, und mehrere solcher Zellfasern durch Reiben sich zu dickeren Fasern vereinigen lassen. Wir sehen also Fasern von verschiedener Dicke entstehen. Ferner ist bemerkenswert, daß diese Fibrillen nicht an Zellgrenzen gebunden zu sein brauchen. Der Zug kann auf viele aneinandergereihte Zellen wirken und dann entstehen Fibrillen,


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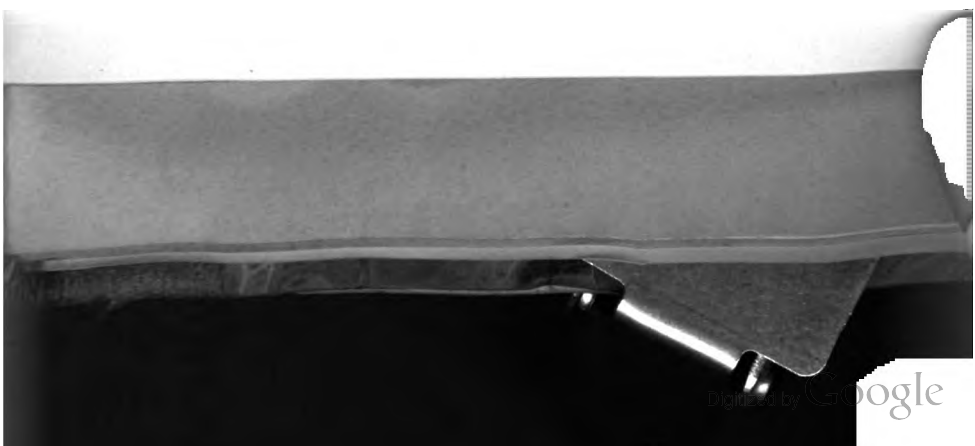
die sich über viele Zellterritorien hin erstrecken. Sowohl die feinere intracelluläre Fibrillation setzt sich kontinuierlich auf Fibrillen benachbarter Zellen fort, wie ganz besonders die aus ganzen Zellen entstehenden Fibrillen. Es ist überraschend, zu sehen, wie oft mit einem Ruck der Zellcharakter aneinandergereihter Zellen völlig schwindet und kontinuierliche Fadensysteme an die Stelle der Zellen treten können. Es ist nachher nur mehr schwer nachzuweisen, daß solche Fasern aus Zellen entstanden sind. Vielfach kann man jedoch noch den Kern in der Mitte einer solchen Faser liegen sehen. Der Kern dieser Zelle ist ein dehnbares, weiches Gebilde, ähnlich wie das Zellprotoplasma. In anderen Fällen bleibt er als eine kleine, runde Kugel im Centrum der Faser liegen. Daß eine Blutzelle lang ausgezogen werden kann, erwähnen schon Halliburton und Hardy. Die vollständige Umwandlung von einzelnen Zellen und Zellhaufen in fibrinähnliche Massen unter vollständigem Schwinden der Zellstruktur ist jedoch ein viel weitgehender Vorgang. Woolridge gab vor 20 Jahren an, er habe den direkten Übergang von Leukocyten von Säugetieren in Massen beobachtet, die von Fibrin nicht unterschieden werden konnten. Woolridge scheint jedoch keine Versuche ähnlich den hier beschriebenen angestellt zu haben. Bei der bloßen mikroskopischen Untersuchung einer Mischung von Zellprodukten mit etwaigen Niederschlägen aus Flüssigkeiten ist es aber, wie ich mich überzeugen konnte, häufig sehr schwer zu bestimmen, wie viel dieser Substanzen direktes Umwandlungsprodukt von Zellen und wie viel Koagulationsprodukte einer vorher gelösten Substanz sind. Die entscheidenden Versuche wurden in diesen Untersuchungen an völlig isolierten Zellen gemacht, und zwar in Fällen, wo es möglich war, isolierte Zellen und Zellhaufen experimentell ohne Beimischung von extracellulärem Fibrin zu erhalten. Die Granula werden unter dem Einfluß von Druck und Zug zuerst kleiner und können sodann ganz schwinden. Man hat oft den Eindruck, als ob es sich bei den Granula um Verdichtungen und Hervorwölbungen des Zellprotoplasmus handelt, die durch Druck und Zug ausgeglichen werden können. (Doch soll damit nicht eine etwaige chemische Verschiedenheit dieser Körner ausgeschlossen werden.) Als Folge der Einwirkung starken Druckes sieht man

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zurückbleiben; diese können dann weiterhin ebenfalls schwinden. Man kann nachweisen, daß Druck sowohl wie Zug dieselbe Wirkung auf Granula haben. Lagen die großen tropfenartigen Gebilde an Stelle der Granula, so scheinen diese spurlos in der durch Zug aus dem Zellprotoplasma gebildeten fibrinähnlichen Masse aufzugehen. Diese großen Tropfen oder stäbchenartigen Gebilde, die besonders in den aufgeschwollenen Zellen entstehen, haben ein sehr ähnliches Lichtbrechungsvermögen wie das Fibrin. Vielfach wurden auch durch Druck Granula aus den Zellen ausgedrückt.

Durch Zugwirkung wurden oft außerordentlich lange Fasern geschaffen. Das beruhte erstens darauf, daß das Protoplasma einer einzigen Zelle sehr stark ausziehbar ist. Das Zellprotoplasma ist unter den gegebenen Umständen ein sehr weiches, plastisches Material. Es genügt, wie man in günstigen Fällen beobachten kann, ein einziger Zug mit dem zweiten Objektträger, um das granuläre Zellprotoplasmas in hyalines fädiges Fibrin zu verwandeln. Zweitens aber verursacht derselbe mechanische Faktor, der das Ausziehen der Zellen bewirkt, daß viele Zellen sich in derselben Richtung in eine einzige kontinuierliche Reihe anordnen. Dasselbe mechanische Moment reiht mehrere Zellen hintereinander auf und verwandelt sie alle in einen einzigen langen Faden. Auch in solchen Fäden verschwinden nun keineswegs immer alle Granula. Oft bleiben einige wenige oder sogar viele in den Fäden eingebettet. Fasern, welche in den Granula eingebettet sind, können nun auch dadurch zustande kommen, daß auf dem Objektträger eine Zelle zerfließt. In dem Augenblick des Zerfließens weichen die Zellgranula in Reihen auseinander und werden durch das fädigwerdende Zellprotoplasma verbunden.

Legen wir einen zweiten Objektträger auf Haufen weicher, geschwollener Blutzellen, welche sich auf einem Objektträger befinden, auf und bewegen unter Druck den oberen Objektträger hin und her, so werden dadurch sehr bald die Zellen in eine klebrige, strukturlose Masse, welche gewöhnlich eine cylindrische Form hat, umgewandelt. Heben wir nun, nach unmittelbar vorausgegangenem Druck den zweiten Objektträger ab, so wird



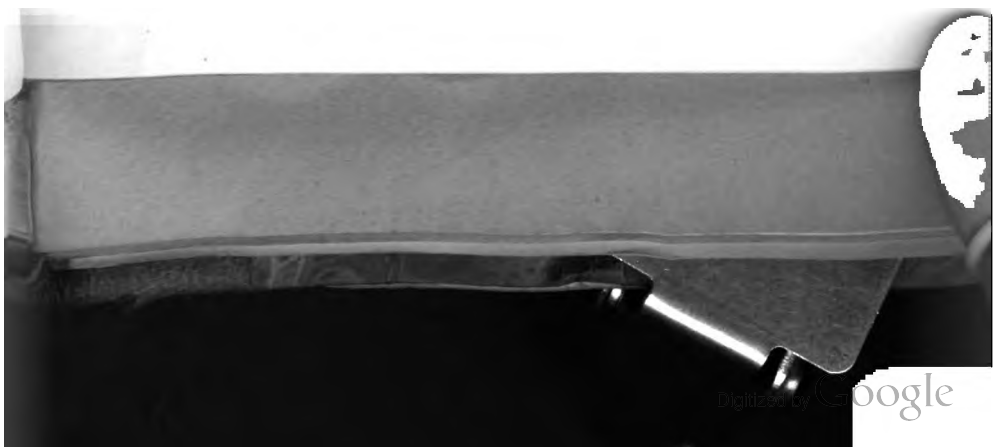
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diese Masse, bei mehrmaligem Wiederholen dieses Vorganges, in faserige Gebilde verwandelt. Wir gelangen so wiederum auf einem Umweg zu Fasern. Häufig tritt zuerst eine Faser in der Mitte der cylindrischen Masse, dann aber auch an anderen Stellen auf. Zuletzt kann die Masse ohne Rest in Fasern aufgehen. Bemerkenswert ist hierbei, daß im Anfang diejenigen Teile der Masse, die schon in Fasern verwandelt sind, sich ganz scharf von den anderen Teilen abheben. Das beruht wohl darauf, daß nur da, wo der mechanische Faktor genügend stark einwirkt, die faserige Umwandlung stattfindet. Und dies ist gewöhnlich nur an scharf umschriebenen Stellen der Fall. Wir können unter dem Mikroskop verfolgen, wie durch bloßen Zug beim Abheben des Objektträgers eine solche Masse zu Fasern ausgezogen wird. Ein Zellcylinder kann hierbei in außerordentlich lange Fasern verwandelt werden. Umgekehrt kann man auch beobachten, daß es möglich ist, eine solche Faser wieder in eine größere cylindrische Masse zusammenzurollen. Auch mit ganz isolierten Zellen, die etwas geschwollen waren, wurden solche Versuche angestellt, und es konnte festgestellt werden, daß das Protoplasma der Zellen selbst alle Eigenschaften, wie Dehnbarkeit, Elastizität und Klebrigkeit hat, die zu diesen mechanischen Veränderungen nötig sind. Nun ist es aber nicht gleichgiltig, mit welchen Zellen diese Versuche ausgeführt werden. Es bestehen darin Unterschiede je nach der Vorbehandlung der Zellen. Am leichtesten können diese Vorgänge festgestellt werden mit Zellen und Zellhaufen, die weich und geschwollen sind, wie dies nach Einwirkung der Adrenalin- oder Atropinlösung zum Beispiel stattfindet. Hier kann man auch ganz sicher sein, daß man nur mit Zellen zu tun hat, die nicht etwa in eine gallertige Hülle eingelagert sind. Aber auch Zellen in Hydrochinon oder in Gelatine sind ein günstiges Objekt. Auch Zellen, die in 3pCt. Formalin aufgefangen wurden, sind im Anfang durchaus fähig, diese Umwandlungen zu zeigen. Schwerer wird dies, wenn die Zellen eine Zeit lang in Formalin sich befunden hatten. Schwieriger ist es auch bei Zellen, die in saturierten Salzlösungen gehalten oder nach vorherigem Erwärmen des Tieres aufgefangen wurden. Die teigartige Ausziehbarkeit des Zellprotoplasmas, die in den erstgenannten Flüssigkeiten so leicht nachweisbar ist,

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gut zum Nachweis dieser Veränderungen. Die Zellen sind in den letztgenannten Fällen zu spröde und etwas weniger klebrig. Doch sind dieselben Umwandlungen auch hier möglich; die Umwandlung zu cylindrischen Massen, das Zerdrücken der Granula, die Ausziehbarkeit der Fasern können auch hier nachgewiesen werden. Hier lösen sich jedoch die einzelnen Zellen leicht von der Gesamtmasse ab. Doch finden sich auch in allen diesen Fällen gewöhnlich einige Zellen, die sich am Boden flach ausbreiten, Zellen mit weit ausgezogenem Protoplasma, „explodierte Zellen". Die Zellform mag in solchen Fällen nur mehr durch den Kern und durch die Granula angedeutet sein. Das Protoplasma solcher ausgebreiteten Zellen zeigt nun dieselbe Dehnbarkeit, wie das in destilliertem Wasser gequollene Zellprotoplasma. Dasselbe kann leicht in Fäden umgewandelt werden. Ebenso ist dieses der Fall bei den Zellen, welche in der ersten Stunde nach dem Ausfließen des Blutes am Boden des Objektträgers hyalin ausgebreitet liegen. Durch Zug kann in diesen Zellen leicht eine fein und grob fibrilläre Struktur produziert werden. Wird dieses Zellnetz mit der Nadel an einer Stelle vom Glase des Objektträgers losgelöst, so retrahiert sich dieses Netz, als ob es unter Spannung gehalten wäre, gerade wie wir dies bei isolierten Zellen beim Ausziehen mit der Nadel in Adrenalinlösungen sahen.

Doch können wir in Bezug auf das hyalin ausgebreitete Zellnetz, das durch die Ausdehnung und Ortsveränderung und zum Teil auch unter Annäherung von Zellen, die vielfach ursprünglich weit entfernt waren, entsteht, nicht so sicher sein, daß hier nicht noch eine transparente gelatinöse Substanz, welche die Zellen umgibt, vorhanden ist, eine Substanz mit zum Teil ähnlichen Eigenschaften, wie das Zellprotoplasma. Aber selbst wenn das in manchen Fällen zuträfe, so würde doch auch hier die Gesamtheit der Zellen selbst die Hauptmasse der dehnbaren Substanz bilden und würde durch ihren Charakter hauptsächlich die Eigenschaften dieser Substanz bedingen. Also auch in diesen Fällen müßte sich das Zellprotoplasma selbst ganz ähnlich verhalten wie die etwa vorhandene gelatinöse extracelluläre Sub-



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stanz. Zudem kann man häufig beobachten, daß beim Zusammendrücken eines Zellhaufens sich Granula leicht ablösen und fortschwimmen. Das könnte wohl kaum der Fall sein, wenn eine gelatinöse Masse die Zellhaufen umgäbe. Es könnten höchstens kleine, retrahierte Fäden außerhalb von Zellen entstehen und in diesem Falle bildeten die Zellen sicherlich die Hauptmasse der gelatinösen Substanz. Ebenso kann man auch aus einem durch Reiben zweier Objektträger entstandenen Zellcylinder ganze Zellen wegdrücken, und zwischen solchen Zellen bleibt keine weitere Substanz übrig.

Legt man sofort nach Auffangen eines frischen Bluttropfens auf einem Objektträger einen zweiten Objektträger auf und zieht diesen, bevor Gerinnung eingetreten ist, unter Druck hin und her, so werden die einzelnen Blutkörperchen zu kleinen Cylindern ausgezogen, in denen sich die Granula in Reihen anordnen. Mehrere solcher Zellcylinder können sich aneinander legen und ein Ganzes bilden. Doch findet hier leicht eine Loslösung der einzelnen Zellen statt, da unter diesen Umständen das Zellprotoplasma sich noch nicht, wie das sehr bald nach dem Verlassen des Tierkörpers stattfindet, unter teilweisem Zerfließen ausgedehnt hatte und da dasselbe daher die für Fibrin charakteristischen Eigenschaften noch nicht gewonnen hatte. Das Protoplasma dieser Zellen ist spröde und die Zellen lassen sich leicht zu Körnchen zerreiben. Auch wenn wir auf Haufen von Zellen, welche länger in Formalin lagen oder auf Haufen von vorher erwärmten Zellen mit einem zweiten Objektträger einen Druck ausüben, wird oft eine reihenweise Anordnung der Granula dieser Zellen sichtbar. Diese durch die Granula markierten Fibrillen können durch mehrere Zellen hindurchgehen, so daß anscheinend die verschiedenen Zellen ein Ganzes bilden.

Die Elastizität dieser durch mechanische Beeinflussung entstehenden fadenförmigen Gebilde ist verschieden. Diejenigen Gebilde, die aus nicht gequollenen Zellen entstehen, zeigen wenig Elastizität, diejenigen hingegen, die aus ausgeflossenem Zellprotoplasma oder aus sich weit ausbreitenden Zellen entstehen, sind elastisch. Eine Bedeutung kommt mechanischen Zugwirkungen wohl auch in dem Falle zu, wenn direkt aus dem bei der Auflösung von Zellen gleichzeitig mit den Granula aus-

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fließendem Zellprotoplasma Fäden entstehen, welche die Granula einschließen. Man kann unter Umständen, wenn man Hummerblut direkt in destilliertem Wasser auf dem Objektträger auffängt, beobachten, daß der Zusammenhang zwischen Zellen, die sich in einiger Entfernung voneinander befinden, dadurch zustande kommt, daß dazwischen liegende Zellen sich zu im Anfang strukturlosem Protoplasma umwandeln. Der Kern allein bleibt dabei in der Mitte der Masse liegen als das einzige Strukturelement; ein wenig Protoplasma mag denselben umgeben. Unter diesen Umständen geraten auch Granula in die „Intercellularsubstanz". In anderen Fällen tritt aus einzelnen Zellen nur ein Teil des Protoplasmas aus; auch durch dieses Protoplasma können nicht direkt sich berührende Zellen gleichsam aneinander gefesselt werden; ferner tritt auch direkte Agglutination vieler Zellen ein. Als Folge der Bewegung mit einer Nadel oder mit einem zweiten Objektträger kann nun dieses extracelluläre Protoplasma in Fasern verwandelt werden; unter solchen Umständen sieht man nur mehr Fasern, wo kurz vorher Zellen oder Zellreste gelegen waren. Während gewöhnlich solches die Zelle verlassende Protoplasma nur durch in demselben gelegene Körnchen angedeutet wird, ist es doch auch zuweilen deutlich sichtbar. Man kann sich sicher davon überzeugen, daß durch mechanische Einwirkung (Zug) aus dem erst formlosen Protoplasma solcher sich auflösenden Zellen geformte Intercellularsubstanz in der Form eines Systems von langen durchgehenden und von kurzen Fäden, welche Körnchen enthalten, gebildet wird. Natürlich kann man nun nicht daraus, daß man an einzelnen Stellen diese Entstehung der Intercellulärsubstanz nachweisen kann, schließen, daß alle Fasern in dieser Weise gebildet sein müssen.

Es wurde früher darauf hingewiesen, daß beim Hummer gewöhnlich eine deutliche zweite Gerinnung erfolgt, daß bei Limulus aber solche Nachgerinnungen meist nur eine geringe Bedeutung haben. Beim Hummer haben diese sekundär gerinnenden Massen den Charakter einer strukturlosen, transparenten Substanz. Durch Zug kann nun auch in dieser Masse eine Fadenstruktur hervorgebracht werden. Oft ist aber eine Fadenstruktur schon von Anfang an in diesen gelatinösen Massen

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vorhanden, und zwar wahrscheinlich hervorgerufen durch in der gerinnenden Flüssigkeit stattfindende Bewegungen. Auch das Produkt der zweiten Limulusgerinnung kann einen gelatinösen Charakter haben, besteht aber oft aus granulären Massen. Wenigstens bilden sich oft nach Ablauf der ersten Gerinnung granuläre Massen in dem Blutserum von Limulus. Diese granulären Massen können nun ebenfalls durch Reiben mit zwei Objektträgern in hyaline, fibrinähnliche Gebilde umgewandelt werden.

Aber nicht nur von vornherein feste oder halbfeste Substanzen, wie Zellen und gelatinöse Massen, werden durch mechanische Umstände in ihrer Struktur geändert, sondern auch kolloidale Lösungen. Reiben wir einige Tropfen Blutserum eines zu diesen Versuchen verwandten Tieres nach der ersten Gerinnung zwischen zwei Objektträgern, so entstehen ganz ähnliche Cylinder, wie sie sich aus den Zellen direkt durch entsprechende mechanische Einwirkungen herstellen lassen. Auch nachdem im Hummer die zweite Gerinnung stattgefunden, lassen sich noch aus dem austretenden Serum durch Reiben ähnliche Cylinder herstellen, aber nun wohl weniger leicht, wie vorher. Um diese Umwandlung in Lösungen zu bewirken, ist ein größerer Druck nötig, wie bei der entsprechenden Umwandlung des Zellprotoplasmas. Durch den durch Abheben des zweiten Objektträgers entstehenden Zug werden auch hier Fäden verschiedener Dicke hervorgebracht. Auch in filtrierten Eiweißlösungen kann man zwischen zwei Objektträgern durch Reiben und Abziehen der Objektträger dieselbe Wirkung hervorbringen. Es ist hierbei bemerkenswert, daß die durch Zug entstehenden Fäden gewöhnlich um Luftblasen liegen. Aber daraus dürfte wohl kaum der Schluß gezogen werden, daß die Produktion organisierter Gebilde aus Lösungen nicht auf mechanischen Umständen, sondern auf der Verdunstung des Wassers beruht. Denn 1. kann man bei Eiweißlösungen schon durch einmaliges Abheben der Objektträger diese Fäden hervorbringen, 2. kann man auch durch Reiben mit zwei Objektträgern unter Wasser dieselbe Wirkung hervorbringen (wenn vielleicht auch hier Luftwirkung nicht vollständig ausgeschlossen ist), 3. sind die entstehenden Gebilde ganz dieselben wie diejenigen, welche entstehen, wenn man noch

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leibt, hierbei scheidet sich dann das Fibrin in Form von Cylindern und von Fasern aus. 4. Findet man auch die aus Zellen gebildeten Fasern um Luftblasen angeordnet, weil die Luftblasen eben die Stelle andeuten, an der die stärkste mechanische Beeinflussung der Zellen oder der Kolloide stattgefunden hat. Bei Eiweißlösungen ist es schon seit langem bekannt, daß durch Schlagen derselben Trübungen entstehen, die auf der Bildung fibrinähnlicher Gebilde beruhen. Durch Reiben mit zwei Objektträgern oder durch Schlagen der Flüssigkeit mit einer Nadel auf dem Objektträger kann man leicht die Entstehung solcher Gebilde mikroskopisch verfolgen. Mit Blutserum von Kaninchen gelang es mir nicht, in einwandsfreier Weise die Bildungen dieser Strukturen nachzuweisen; auch Ramsden konnte durch Schlagen des Blutserums keine Wirkung erzielen. Täuschungen können bei Verwendung zweier Objektträger dadurch entstehen, daß Glassplitter, welche vom Objektträger abgerieben werden, oder dem Objektträger zufällig aufliegende Fasern anderer Art mit den aus den kolloidalen Substanzen entstehenden Gebilden verwechselt werden. Es kann beobachtet werden, daß die aus Eiweiß entstehenden Fasern, sobald sie Luftblasen nahe kommen, an der so gebildeten Grenzschicht zwischen Luftblase und Flüssigkeit hängen bleiben. Solche Fasern können auch Luftblasen einschließen. Oft haben diese aus kolloidalen Lösungen entstehenden Gebilde ebenfalls ein körniges Gefüge, welches durch Druck ausgeglichen werden kann. Mit Gelatinelösungen lassen sich ähnliche Fäden auf mechanische Weise nicht herstellen.

Es ist sicherlich eine bemerkenswerte Tatsache, daß aus Zellen durch relativ geringen Druck und Zug anscheinend Gebilde mit ähnlichen physikalischen Eigenschaften hervorgebracht werden, wie aus kolloidalen Lösungen von Hühnereiweiß und Bluteiweißstoffen der Arthropoden. Durch einen kleinen, für beide gleichen mechanischen Eingriff werden aus Zellen und aus in kolloidaler Lösung befindlichen Zellprodukten dieselben Gebilde geschaffen. Hierdurch wird bewiesen, daß der Eiweißcharakter der Zellsubstanzen für einen Teil der morphologischen Charaktere der Zelle allein in Betracht kommt, insbesondere


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für den unter mechanischen Bedingungen auftretenden fibrillären Charakter gewisser protoplasmatischer Gebilde.

Über die zweite Gerinnung des Hummerblutes.

Bisher wurde die Gerinnung als ganzes untersucht. Nun ist es aber möglich, eine bestimmte Phase der Gerinnung isoliert zu untersuchen, nämlich die zweite (gelatinöse) Gerinnung in dem Plasma, das nach Ablauf der ersten fädigen Gerinnung übrig bleibt. Genauere Versuche scheinen hierüber nicht vorzuliegen. Einzelne Autoren begnügen sich damit, die Frage zu diskutieren, ob diese zweite Gerinnung kontinuierlich mit der ersten stattfindet oder nicht. Halliburton bejaht dies. Nur Frédericq[1]) gibt kurz an, daß Erwärmen auf ungefähr 50° C. sowie Zusatz gewisser saturierter Salzlösungen (Mg SO4) die zweite Gerinnung hindert. Im folgenden sollen nun die Resultate einiger Versuche mitgeteilt werden, die alle am Hummer, der sich hierzu am besten eignet, angestellt wurden. Es ergab sich gleich im Anfang, daß wenn man nach dem Herausfließen des Blutes das fädige Gerinnsel durch Schütteln schnell sammelt und mit Nadeln entfernt, die Gerinnung auf längere oder kürzere Zeit gehemmt oder auch ganz aufgehoben werden kann. Jedoch gerinnt das Blut, zwar nicht immer, aber doch häufig auch unter diesen Umständen, wenn auch später als unter gewöhnlichen Umständen, nämlich ohne Herausnahme des fädigen Gerinnsels. Das hängt zum Teil sicher davon ab, daß es unter diesen Umständen nicht möglich ist, alles Fadengerinnsel zu entfernen. Untersucht man die sich am Boden bildenden Gerinnsel auch nach möglichst sorgfältigem Entfernen des fädigen Koagulums, so sieht man sehr häufig unter dem Mikroskop im Centrum der sich bildenden kleinen Gerinnsel einen kleinen Haufen von Blutzellen. Aber es kann nicht ausgeschlossen werden, daß nicht auch bei völliger Herausnahme aller Zellen doch Gerinnung eintreten kann. In vielen Fällen jedoch bleibt die zweite Gerinnung auch trotz der nur unvollständigen Entfernung aller Gerinnsel aus. Es ist möglich, daß allein schon das durch die Auflösung so vieler

[1]) Frédericq: Bulletin de l'Académie Royale de Belgique 3. serie tome I.

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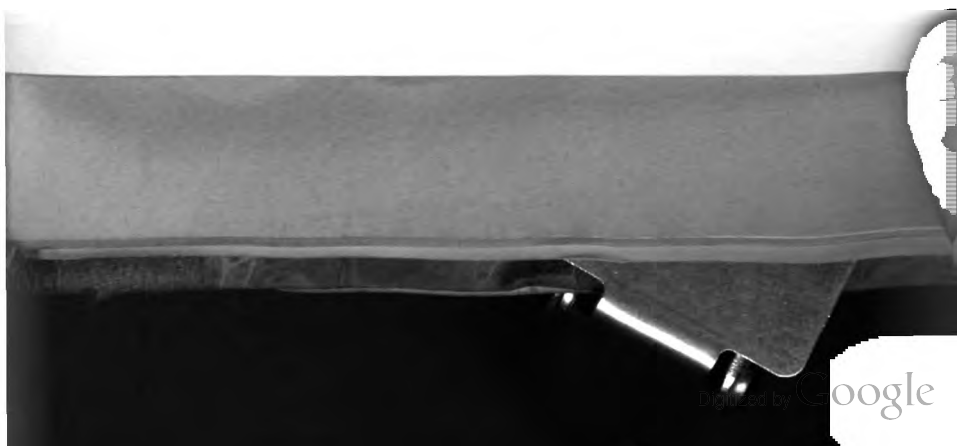
auch bei vollständiger Entfernung der Zellen eine Gerinnung zu veranlassen. Später fand ich dann, daß durch Zusatz von Wasser zu dem Blut während der Bildung des Fadengerinnsels und durch nachfolgende Filtration die Gerinnung mit größerer Sicherheit auf längere Zeit oder ganz aufgehoben werden kann. Es wurde in solchen Versuchen etwa 25—100 pCt. Wasser dem Blute zugefügt. Es folgt aus diesen Versuchen, daß durch die Verdünnung des Blutes nicht nur die Menge des sich bildenden gelatinösen Gerinnsels relativ, nämlich im Verhältnis zu der nun vergrößerten Flüssigkeitsmenge herabgesetzt wurde, sondern, daß die Fibrinausscheidung auch absolut für eine bestimmte Zeit verringert wurde. Mit diesem Plasma, das ohne weiteren Zusatz nun eine Zeit lang spontan nicht gerann, wurden dann weitere Versuche meist in der Weise angestellt, daß je 4 oder 5 ccm desselben in eine Anzahl Schälchen gegossen wurden. Einigen dieser Schälchen wurden sodann abgemessene Mengen gewisser Lösungen zugesetzt. Ferner wurden hierauf in gewisse Schälchen bestimmte feste Substanzen gelegt, insbesondere kleine Stückchen des bei der ersten Gerinnung gebildeten fädigen Fibrins. Hierbei wurde darauf geachtet, daß annähernd gleiche Volumina dieser Substanzen zugesetzt wurden, so daß wir in einer Versuchsserie annähernd quantitativ untereinander vergleichbare Resultate erhielten. Es zeigte sich nun, daß Zusatz von etwa zwei bis drei Stückchen des fädigen Hummerfibrins zu dem Serum einen sehr starken Einfluß auf die Gerinnung hatte. Während ohne Zusatz dieses Fibrins die Gerinnung gar nicht oder oft erst nach mehreren Stunden oder erst am nächsten Tage eintrat, begann in gewöhnlichen Fällen bei Zusatz dieses Fibrins die Gerinnung viel früher einzutreten. Die Schnelligkeit, mit der das Fibrin die Gerinnung bewirkte, war verschieden. Der Unterschied gegen das Kontrolplasma, welches kein Fibrin erhielt, war aber immer sehr ausgesprochen. Bemerkenswert war ferner, daß der Einfluß dieses Fibrins ein durchaus lokalisierter war. Die Gerinnung begann immer direkt um das Fibrinstück und rückte von hier aus konzentrisch vor. Zuerst bildete sich meist eine Gallerte auf der unteren Seite des Fibrinstückes.


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Infolge dessen klebte dieses am Boden des Schälchens fest. Hier bewirkte also eine sich aus der Flüssigkeit ausscheidende Substanz das Festkleben. Aber das Fibrin selbst besteht aus einer klebrigen Substanz, so daß dasselbe auch in reinem Wasser festkleben kann. Fibrin ist nun nicht die einzige Substanz, die eine solche Wirkung hat. Muskelstücke des Hummers wirken ungefähr gleich stark. In diesen Versuchen wurde der Muskel sorgfältig gewaschen und gebürstet, um etwa anhaftendes Blut zu entfernen. Nerv des Hummers hatte keine ausgesprochene Wirkung, doch war die hierzu benutzte Quantität der Substanz peripherischer Nerven sehr gering. Fibrin des Kaninchen- oder Rattenblutes oder Froschmuskel waren ohne Wirkung. Auch in diesen Fällen wurden diese Substanzen vorher ausgewaschen oder sie wurden vor dem Versuch zwei Stunden lang in Hummerplasma ausgelaugt, um etwaige in dem Fibrin anderer Tiere befindliche, die Gerinnung hemmende Stoffe zu entfernen. Gleich behandeltes Hummerfibrin war wirksam, Kaninchen- und Rattenfibrin waren unwirksam. Wurde zu dem Hummerplasma, in welchem das Kaninchenfibrin gelegen hatte, nachträglich Hummerfibrin zugefügt, so gerann das Plasma, also waren die Gerinnung hemmende Substanzen aus dem Säugetierfibrin nicht extrahiert worden. Kleine Pankreasleberstücke des Hummers verhinderten, wie zu erwarten, die Gerinnung vollständig. Wurden wirksame Fibrinstücke des Hummers zehn Minuten oder länger in absoluten Alkohol gelegt, sodann gewaschen und getrocknet, so hatten sie ihre Wirksamkeit fast vollständig eingebüßt. Eine Spur Fibrin vermochte sich jedoch zuweilen auch um solche Stücke noch abzulagern. Kontrolversuche zeigten, daß extrahierter, mit dem Serum sich vermischender Alkohol nicht die Ursache dieses Verlustes an Wirksamkeit sein konnte. Fibrinstücke, welche über Nacht oder drei Tage lang in Chloroformwasser gehalten wurden, konnten noch recht wirksam sein, wenn auch nicht in demselben Grade, wie frisches Fibrin. Fibrin, das fünf Tage in Chloroformwasser gehalten war, hatte seine Wirksamkeit in bedeutendem Maße eingebüßt. Zusatz von 1 ccm Chloroformwasser zu 4 oder 5 ccm Serum oder von einigen Tropfen reinen Chloroforms hatte keinen stärkeren Einfluß als Zusatz von Wasser. Erhitzen des Fibrins im Wasserbad auf 46 bis

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großen Teil, aber nicht vollständig auf, Erwärmen auf 53° bis 54° oder auch nur auf 51° während 40 Minuten hob die Wirksamkeit ganz oder fast ganz auf. Erwärmen des Plasmas auf 46 bis 50° während 30 Minuten verhinderte die spontane Gerinnung des Plasmas (die ja vielleicht nur durch darin verteilte Zellen oder Zellteile herbeigeführt wird). Setzte man zu derartig erwärmtem Plasma frisches Fibrin, so gerann dieses Plasma fast ebenso schnell, wie normales Plasma. Doch zeigte sich häufig eine sehr geringfügige Verringerung der Geschwindigkeit der Gerinnung dieses Plasmas im Vergleich zu nicht erwärmtem Plasma. Zufügen von 1 ccm Glycerin zu 4 ccm Plasma hatte keinen spezifisch hemmenden Einfluß. Solches Plasma gerann ungefähr ebenso schnell wie reines Plasma oder Plasma, dem 1 ccm 0,6 pCt. Natriumchloridlösung zugesetzt war. Auch Zusatz von 1 ccm Lösung von reiner Gelatine war ohne spezifischen Einfluß. Nach den früher geschilderten Versuchen lag die Möglichkeit vor, daß Gelatine hemmend wirken könnte. Bloßer Zusatz von 1 ccm Wasser oder von 1 ccm 0,6 pCt. bis 4 pCt. Natriumchloridlösung zu 4 ccm Plasma hatte in den meisten Fällen eine schwach hemmende Wirkung, doch kam es vor, daß solche Plasmamischungen sogar etwas schneller gerannen, wie das reine Plasma; möglicherweise spielten hierbei Zufälligkeiten, wie verschiedener Gehalt der Mischung an Blutzellen, eine Rolle. Alle diese Versuche, wie auch die meisten der folgenden Versuche wurden unter Zusatz der Fibrinflocken zu der Flüssigkeit ausgeführt. Hingegen hatte der Zusatz von 1 ccm 1 pCt. Kaliumcyanid oder 16 pCt. Witte-Peptonlösung oder saturierter Harnstofflösung einen entschieden, wenn auch oft nur für einige Zeit hemmenden Einfluß; später, z. B. nach sechzehn Stunden, zuweilen auch früher, vermag dennoch Gerinnung aufzutreten. Auch tritt die Einwirkung gewöhnlich erst nach dem Ablauf der ersten halben Stunde zutage. Die Reihenfolge der Stärke der gerinnungshemmenden Wirkung dieser Substanzen ist die folgende: Kaliumcyanid wirkt am schwächsten, Harnstoff steht in der Mitte und Pepton wirkt am stärksten. Stellt man jedoch die Versuche in der Weise an, daß man die Fibrinstücke zuerst für einige Zeit in die Kaliumcyanid-, Harnstoff- und Peptonlösungen legt, diese Fibrinstücke sodann aus-


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wäscht und erst dann in Schälchen, welche das Blutplasma enthalten, legt, so ist die Reihenfolge des Wirksamkeitsverlustes gerade die umgekehrte, wie in den vorigen Versuchen. Die Stücke, die eine halbe bis zwei Stunden in Peptonlösungen lagen, sind fast gerade so wirksam, wie frische Fibrinstücke, sogar Stücke, die über Nacht in Pepton lagen, können noch sehr starke Wirkung zeigen, wenn sie auch wohl in anderen Fällen schon einen bedeutenden Teil ihrer Wirkung verloren haben können. Es muß darauf geachtet werden, daß das Pepton während einiger Stunden aus dem Fibrin ausgewaschen wird, bevor dasselbe dem Plasma zugefügt wird, sonst wirkt das in das Plasma austretende Pepton gerinnungshemmend. Stücke, die eine halbe bis zwei Stunden in der Harnstofflösung lagen und dann ausgewaschen wurden, haben den größten Teil ihrer Kraft verloren, zwölfstündiges Liegen vernichtet dieselbe völlig. 1 pCt. Kaliumcyanid vernichtet die Wirksamkeit des Fibrins meist schon in einer halben Stunde völlig. Natürlich kann auch in solchen Schälchen soviel Gerinnung eintreten, wie in Kontrolschälchen, welche gar kein Fibrin enthalten, stattfindet. Liegt ein Stück Fibrin zwei Stunden lang in einer 1 pCt. Kaliumcyanidlösung, so wird das Fibrin opak. Die Verschiedenheit der Wirkung der drei Stoffe, die sich in diesen Versuchen kund tut, kann in mehrfacher Weise gedeutet werden. Das soll in einer späteren Arbeit erörtert werden.

Während wir in früheren Versuchen sahen, daß die Oxalate nicht stärker wirken, wie eine größere Reihe anderer Salze, welche Calcium nicht präzipitieren, können wir bei der zweiten Gerinnung des Hummers einen deutlichen Einfluß der Oxalate wahrnehmen.

1 ccm 2 pCt. Kaliumoxalat oder fünf Tropfen 10 pCt. Kaliumoxalat zu 4 ccm Plasma hinzugefügt, hindern die Gerinnung völlig, $\frac{1}{2}$ ccm 2 pCt. Kaliumoxalat verzögert die Gerinnung deutlich. Umgekehrt hatte der Zusatz von 5 Tropfen, 1 ccm oder 2 ccm einer 2 pCt. Calciumchloridlösung in einer Serie von Versuchen einen deutlichen, wenn auch nicht starken, fördernden Einfluß auf die Gerinnung, im Vergleich zu Kontrolversuchen mit Zusatz von 1 ccm schwacher Natriumchloridlösung. Wahrscheinlich ist Calcium im Plasma schon in genügender Menge vorhanden, so

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fördernden Einfluß haben kann.

Größere Versuchsreihen über die Wirkungen von Salzen sind bisher noch nicht angestellt worden, sollen aber noch ausgeführt werden. Doch machte ich im Anschluß an die Versuche mit Harnstoff Versuche über die Wirkung von Ammoniumchlorid. Es zeigte sich hierbei, daß dieses Salz, verglichen mit Natriumchloridlösungen, einen stärker hemmenden Einfluß auf die Gerinnung ausübt.

Während Zusatz von 1 ccm 16 pCt. Natriumchloridlösung oft nur einen unbedeutend (zuweilen allerdings auch einen etwas stärkeren) hemmenden Einfluß ausübt, haben 7 Tropfen einer 16 pCt. Ammoniumchloridlösung oder 1 ccm einer nur 4 pCt. Ammoniumchloridlösung eine weit stärkere Gerinnungshemmende Einwirkung. 1 ccm 16 pCt. Ammoniumchlorid hindert die Gerinnung fast ganz. 1 ccm 16 pCt. Acetamidlösung hat nur einen schwach hemmenden Einfluß. Zusatz einiger Tropfen Ammoniak hindert die Gerinnung vollständig, doch beruht dies wahrscheinlich auf Alkaliwirkung.

Läßt man die Blutplasmamischungen 24 Stunden lang in einem warmen Zimmer stehen, so tritt allmählich Fäulnis der Flüssigkeit ein. Die Fäulnis schließt nun das Eintreten der Gerinnung nicht aus, wenn sie dieselbe auch erschweren mag.

Nachdem die zweite, gelatinöse Gerinnung stattgefunden, ist es möglich, ein Serum aus der resultierenden geronnenen Masse zu gewinnen. Dieses Serum gerinnt nun, wenn es mit fädigem Fibrin versetzt wird, nicht mehr, obwohl, wenn es zwischen zwei Objektträgern gerieben wird, noch Fasermassen aus demselben gewonnen werden können.

In einer Reihe von Versuchen wurde geprüft, ob das um das erste, nämlich das fädige Gerinnsel sich bildende gelatinöse Fibrin ebenso stark wirkt, wie das ursprüngliche fädige Fibrin. Es zeigte sich, daß das gelatinöse Gerinnsel, welches sich bald nach dem Ausfließen des Blutes und nach der Bildung der Fibrinfäden um das fädige Fibrin bildet, ungefähr ebenso stark wirkt wie das erste, die Zellen enthaltende Gerinnsel, daß aber


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das sich erst nach einiger Zeit in der Peripherie ansetzende gelatinöse Gerinnsel erheblich schwächer wirkt.

Mit verschiedenen Fremdkörpern (feste Gelatine, Holzkohle, Kartoffelstücke, gekochte Muskel, Karmin) wurden Versuche angestellt, um festzustellen, ob Fremdkörper als solche die Gerinnung beschleunigen. Es ergab sich, daß sie wirkungslos waren. Jedenfalls konnte eine deutliche Wirkung nie beobachtet werden. Von Interesse ist auch, daß Platinmohr ganz unwirksam war. Es ist möglich, daß in dem Augenblick, in dem sich das Fibrin ohnedies ausscheidet, der Ort der ersten Ausscheidung durch Fremdkörper beeinflußt wird. Darauf weist vielfach die Tatsache hin, daß im Gegensatz zu der Regel, daß im Centrum kleiner Gerinnsel Häufchen von Blutzellen liegen, in einigen Fällen solche nicht mikroskopisch gefunden werden konnten, wohl aber zum Beispiel ein zufällig in die Flüssigkeit geratener Baumwollfaden. Doch mögen zu dieser Zeit einzelne Zellen schon so weit aufgelöst gewesen sein, daß ihr Vorhandensein nicht mehr erkannt wurde. Blutplasma, welches in Glasröhren mit geringem Durchmesser gegossen wurde und so überall in Berührung mit festen Körpern war, gerann nicht früher wie in offenen Schalen gehaltenes Serum.

In dem in offenen Schalen gehaltenen Blutplasma bildet sich gewöhnlich ein Oberflächenhäutchen, und zwar geht auch dieses meist radiär von den eingelegten Fibrinflocken aus. Wenn wir Plasma eine halbe Stunde lang gegen eine unebene Fläche schütteln, so scheidet sich wie bei mechanischer Bewegung auf dem Objektträger Fibrin in makroskopisch erkennbaren Häutchen aus.

Es wurde auch eine Reihe von Versuchen angestellt, in denen versucht wurde, die Wirkung des Zellfibrins durch wässerige Extrakte zu ersetzen. Sowohl frisches, wie in absolutem Alkohol gehaltenes Fibrin wurde stunden- bis wochenlang mit Chloroformwasser extrahiert. Mit Sicherheit konnte ich mich nun nicht von der Wirksamkeit solcher Extrakte überzeugen. 1 ccm des Extraktes zu 4 ccm Plasma hinzugefügt zeigte in den meisten Fällen eine ganz unbedeutende Beschleunigung der Gerinnung im Vergleich zu Plasma, dem 1 ccm 0,6 pCt. Natriumchloridlösung zugefügt worden war. Der Unterschied war jedoch

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unbedeutend und die Wirkung des Extraktes stand der Wirkung des Fibrins an Stärke außerordentlich nach. Auch Extrakte, welche durch mehrstündiges Ausziehen des Fibrins ohne Chloroformzusatz hergestellt wurden, gaben kein besseres Resultat. Vielleicht dürften andere Extraktionsmethoden sich wirksamer erweisen. Halliburton gibt an, daß ein Extrakt von in Alkohol präzipitiertem und dann gepulvertem Säugetierblut Gerinnung in dem mit Wasser verdünnten Magnesiumsulfatblut von Arthropoden hervorrief. Mir gelang es nicht, auf diese Weise Gerinnung herbeizuführen. Ein solches Resultat würde auch nicht in Einklang stehen zu der oben zutage getretenen Unwirksamkeit von Kaninchen- und Rattenfibrin.

Als ein Beispiel möge folgendes Versuchsprotokoll angeführt werden:

25. Nov. II. Versuch. 2.50 Uhr nachmittags Hummerblut mit 50 pCt. Wasser versetzt, die Fasergerinnsel durch Filtration entfernt, das Plasma in Schälchen gegossen.

1. 4 ccm Plasma + 3 Fibrinstücke, die vorher 3 Stunden in Wasser gelegen hatten.
2. 4 „ + 3 Fibrinstücke, die vorher 1 Stunde in 16 pCt. Pepton, sodann 2 Stunden in Wasser gelegen hatten.
3. 4 „ + 3 Fibrinstücke, die 1 Stunde in sat. Harnstofflösung, 2 Stunden in Wasser gelegen hatten.
4. 4 „ + 3 frische Fibrinstücke.
5. 4 „ + 3 Fibrinstücke von Kaninchenblut. (Fibrin vor dem Versuch gewaschen.)
6. 4 „ + 3 Hummerfibrinstücke, welche vorher 40 Minuten lang auf 50 bis 51 ° erwärmt worden waren.
7. 4 „ + 3 Hummerfibrinstücke, die vorher 5 Tage in Chloroformwasser gehalten waren.
8. 4 „ ohne Zusatz.
9. 4 „ + 3 Stücke des gelatinösen zweiten Koagulums (3 Stunden alt).
10. 4 „ + Platinschwamm (den Boden des Schälchens bedeckend).
11. 4 „ + 1 ccm 0,6 pCt. NaCllösung.
12. 4 „ + 1 ccm Extrakt des fädigen Fibrins in Chloroformwasser.

Um 3.30 nachmittags: 1. fast ganz geronnen, 2. um das Fibrin kleine, sagokornähnliche Gerinnsel, das Fibrin klebt fest, 3. ungeronnen, Fibrinstücke locker, 4. ¾ geronnen, 5. nicht geronnen, Stücke nicht fest, 6. nicht geronnen, Stücke nicht fest, 7. Plasma nicht geronnen, Stücke nicht fest, 8. nicht geronnen, 9. Stücke Fibrin fest, nicht geronnen, 10. nicht geronnen, 11. und 12. nicht geronnen.

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Um 4.20 nachmittags: 1. und 4. ganz geronnen, 2. fast ganz geronnen, 3. ungeronnen, alle anderen mit Ausnahme von 9. ebenfalls ungeronnen, in 6. klebt Fibrin fest, in 9. Fibrin klebt fest, eine Spur Gerinnung.

5 Uhr nachmittags: 9. ganz geronnen, sonst kein Wechsel.

Am nächsten Morgen, 9 Uhr vormittags: 3. und 5. nicht geronnen, 8. nicht geronnen, 10. 11. 12. nicht geronnen, 7. geronnen, 6. ¼ geronnen; sonst keine Veränderung.

Wir können nun alle diese Befunde durch die Annahme erklären, daß in dem Fadengerinnsel ein Gerinnung bewirkendes Ferment oder Proferment vorhanden ist, und ferner durch die Annahme, daß, da dieses Gerinnsel zum überwiegenden Teil aus Zellen des Blutes besteht, ein solches Ferment oder Proferment in den Blutzellen vorhanden ist. Wir wiesen weiterhin eine streng lokalisierte Wirkung dieses Fermentes nach. Direkt um die Fibrinflocken lagert sich das Fibrin ab. Es muß daher angenommen werden, daß das Ferment nur langsam in die Flüssigkeit diffundieren kann. Trotz dieser Schwierigkeit, sich auszubreiten, müßte aber dennoch das Ferment im stande sein, durch die gelatinöse Substanz, die sich um das Fibrin bildet, zu diffundieren, obwohl aus neuen Versuchen hervorzugehen scheint, daß sogar Seifen mit Gelatine vermischt nicht aus der Gelatine zu diffundieren vermögen. Diese lokalisierte Anlagerung des gebildeten Fibrins erinnert sehr an Krystallisationsvorgänge. Bedienen wir uns der Hypothese einer Fermentwirkung, so müßte, wie wir aus den beim Erhitzen des Plasmas gewonnenen Resultaten schließen können, auch das Plasma eine geringe Menge Ferment oder Proferment enthalten. Das Ferment wurde schon teilweise zerstört durch Erhitzen auf 50—51 °. Es besteht eine quantitative Beziehung zwischen Ferment und Gerinnung, da die Gerinnung um so schneller vor sich geht, eine je größere Anzahl Fibrinstücke in das Serum gelegt wurde.

Während absoluter Alkohol zur Darstellung des Säugetier- und nach Halliburton auch des Arthropodenfibrinfermentes benutzt wird, sehen wir, daß eine kurze Einwirkung von absolutem Alkohol auf das zerkleinerte Gerinnsel diesem bereits nach sehr kurzer Zeit seine Gerinnung bewirkende Eigenschaft fast ganz raubt. Hardy nahm an, daß das Ferment in den Granula der Zellen sei, weil diese während der Gerinnung teil-

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sich, so daß kein Grund vorliegt, den Granula eine besondere Bedeutung für die Gerinnung zuzuschreiben. Eine solche Bedeutung dürfte auch deswegen den Granula nicht zukommen, weil wie wir sahen, Hummermuskelstücke, die keine Granula haben, ganz ebenso wirken wie das hauptsächlich aus Zellen bestehende Fadengerinnsel. Während aber in Bezug auf andere Zellarten oder auf Produkte derselben, so lange sie derselben Tierart angehören, ein Mangel an Spezifizität der Fermentwirkung vorliegt, finden wir, daß Fibrin (welches ebenfalls Blutzellen einschließt) sowie Muskel von Kaninchen und Frosch wirkungslos waren. Hier liegen offenbar ähnliche Verhältnisse vor, wie sie sich in den Präzipitinreaktionen von Eiweißstoffen ergaben. Die Reaktionen sind meist mehreren Eiweißstoffen derselben Tierart gemeinsam, sind aber verschieden für die anscheinend identischen Eiweißstoffe, welche von verschiedenen, nicht nahe verwandten Tierarten gewonnen wurden. Auch die Tatsache der gerinnungerregenden Wirkung von Muskel steht nicht isoliert da, indem Delezenne[1]) fand, daß die Berührung mit der Musklatur das Vogelblut zur Gerinnung bringt. Während es aber möglich ist, Vogelblut, falls die Berührung mit Muskel vermieden wird, lange flüssig zu erhalten, trotz der Anwesenheit der weißen Blutkörperchen, ist das beim Hummerblut meist nicht möglich, da in den Blutzellen ein wie Muskel wirkender Stoff vorhanden ist. Doch fand auch Delezenne einen die Gerinnung, wenn auch nur in geringerem Grade, beschleunigenden Einfluß der Leukocyten. In Bezug auf die Vielheit der Fibrinfermente liegen Beobachtungen von Bernheim-Karrer[2]) vor, welche für eine Verschiedenheit der in der Frauen- und Kuhmilch enthaltenen Fibrinfermente sprechen. Alle diese Beobachtungen zeigen, daß in diesen Tatsachen ganz allgemein giltige Gesetzmäßigkeiten vorzuliegen scheinen.

[1]) C. Delezenne, Recherches sur la coagulation du sang chez les oiseaux, Archiv de physiol. norm. et pathol. 1897. T. IX.

[2]) Bernheim-Karrer, Untersuchungen über das Fibrinferment der Milch. Centralblatt f. Bact. Bd. 31. 1902.


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Über die bei der Gerinnung des Blutes in Betracht kommenden Faktoren.

Nachdem wir nun eine Reihe einzelner Tatsachen mitgeteilt haben, müssen wir im Zusammenhang einige daraus sich ergebenden Schlußfolgerungen besprechen. Folgende Faktoren kommen bei der Blutgerinnung wesentlich in Betracht: 1. das Zusammenkleben der Zellen. Wir haben hierauf an vielen Stellen hingewiesen. Ganz isoliert, ohne Beteiligung anderer Faktoren können wir es z. B. beobachten beim Auffangen des Blutes in gewöhnlichem Wasser, in Gelatine oder in Adrenalin. In diesem Falle liefert das Cytoplasma selbst die klebrige Substanz. Wir haben es hier mit einer spontan eintretenden Erscheinung zu tun, die wohl identisch ist mit der Agglutination, die bei Bakterien und den verschiedensten Tierzellen hervorgerufen wurde. Die Gerinnung des Arthropodenblutes beruht zum Teil auf einer spontan außerhalb des Körpers eintretenden Agglutination der Blutzellen. 2. Wie die Zellen zusammenkleben, so klebt auch das aus an einer Stelle geborstenen, scheinbar aber oft intakten Zellen ausgetretene Zellprotoplasma sowie das Protoplasma völlig aufgelöster Zellen zusammen und bildet eine gelatinöse Masse. 3. Während der ersten Stunden der Koagulation breiten sich die Blutzellen in Berührung mit festen Körpern aus, so daß sie bald ein feines Netz sich berührender, weit ausgebreiteter Zellen bilden. 4. Wir sahen nun, daß aus Zellen und aus Zellen ausgetretenem Protoplasma ein vollständiges, von dem extracellulär entstehenden Fibrinnetz nicht zu unterscheidendes Fasernetz gebildet werden kann; wir sahen, daß an vielen Stellen, an denen anscheinend extracellular entstandene Fasern liegen, ein in der Mitte gelegener Kern oder eingeschlossene Granula den Ursprung der Faser aus Zellen andeuten; wir wiesen ferner nach, daß größere Zellhaufen, welche unter bestimmten Bedingungen lediglich durch Agglutination von Zellen entstehen können, durch mechanische Einwirkung in eine von Fibrin nicht unterscheidbare Masse umgewandelt werden können; weiterhin, daß in manchen Zellhaufen die Zellen durch Druck sich loslösen lassen, ohne daß ein extracelluläres Fasernetz oder eine extracelluläre Gallerte übrig bliebe, und daß wir daher annehmen können, daß das Zellprotoplasma selbst die fibrinähnliche Substanz

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Fibrin dieselben physikalischen Eigenschaften besitzt, wie das extracellulär gebildete Fibrin, ferner daß viel Protoplasma aus Zellen ausfließen kann, und daß sogar ganze Zellen zerfließen können, und daß solches Protoplasma ebenfalls eine fibrinähnliche Substanz liefert. Doch kann ein solcher Ursprung nicht für alle Fälle nachgewiesen werden, da man eine Zelle gerade im Zerfließen überraschen muß, um diesen Entstehungsprozeß feststellen zu können, und da dies natürlich nur zuweilen gelingt. Ziehen wir alle diese Tatsachen in Betracht, so können wir den Schluß ziehen, daß ein beträchtlicher Teil allen Fibrins oder der fibrinähnlichen Substanz direkt aus Zellprotoplasma entsteht; es wird aber die Annahme nahegelegt, daß die gesamte gelatinöse Masse diesen Ursprung haben mag. Dieses ist jedoch keineswegs erwiesen. Es dürfte aber wohl möglich sein, diese Annahme später in exakter Weise zu prüfen. Wie dem auch sei, es findet sicher eine Ausscheidung einer vorher in kolloidaler Lösung befindlichen Substanz statt. Wir untersuchten einige Bedingungen dieses Gerinnungsprozesses beim Hummerblute. Aber daß auch bei Limulus und Libinia eine ähnliche Gerinnung einer vorher gelösten Substanz stattfindet, darauf weisen verschiedene Beobachtungen hin. Wir können zum Beispiel unter dem Mikroskop beobachten, daß mit dem Blut gemischte Spermatozoen oder Staubpartikel, die sich sogleich nach dem Ausfließen des Blutes auf dem Objektträger bewegten, plötzlich während der Gerinnung unbeweglich werden, als ob sie von unsichtbaren Fäden festgehalten würden, ohne daß Blutzellen in der Nähe sind. Es gelang ferner, auch in Limulusblut 1—2 Stunden nach dem Ausfließen des Blutes durch Zug mit einer Nadel ein sehr feines Netzwerk sichtbar zu machen, in welchem das gewöhnliche gröbere Fibrinnetz eingebettet lag. Wahrscheinlich entstanden in diesem Fall die feinen Fasern auf Grund der mechanischen Einwirkung aus einer vorher nur halbfesten Masse. Ferner sahen wir, daß auch bei Limulus eine Nachgerinnung des Plasmas stattfinden kann. Diese Tatsachen weisen darauf hin, daß auch hier eine vorher flüssige Masse allmählich fest wird.

Es wurden weiterhin einige Versuche zur Analyse der Faktoren angestellt, welche die Agglutination der Blutkörperchen



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eine sehr stark agglutinierende Wirkung auf Spermatozoen von Arbacia ausübt. Ein bis vier Tropfen Serum zu 6 ccm Meerwasser, welches Spermatozoen enthielt, zugesetzt, bewirkt eine sofortige Agglutination der Spermatozoen in makroskopisch sichtbaren Strängen. Diejenigen Spermatozoen, die nicht agglutiniert werden, können lebhaft beweglich bleiben. Diese Wirkung des Limulusserums geht verloren, nachdem es oft filtriert worden war. Hummerserum hat entweder keine agglutinierende Wirkung oder eine bedeutend schwächere. Ebenso wirkt Limulusserum agglutinierend auf die Blutkörperchen der Schildkröte, wenn eine Blutkörperchenaufschwemmung und Limulusserum im Verhältnis von 1 : 1 oder 10 : 1 gemischt werden. Setzt man zu einem Schälchen Seewasser, in dem sich Larven von Arenicola befinden, einige Tropfen Limulusserum, so hört die ciliäre Bewegung auf. Die Larven kleben mit einem Ende am Boden des Gefäßes fest, die muskuläre Bewegung aber bleibt bestehen, die Larven sondern einen Cocon ab und sterben nach einigen Stunden. Nach Zusatz von einigen Tropfen Hummerserum oder von einigen Tropfen des Magendarmsaftes der Libinia bleibt die ciliäre Bewegung erhalten; die Larven schwimmen umher. Fügt man 4—5 Tropfen Limulusserum zu 5—6 ccm Seewasser, in dem Blastulae oder Plutei von Arbacia schwimmen, so hört die Bewegung dieser sofort auf. Hummerserum hat eine viel schwächere Wirkung, und der Verdauungssaft in Libinia ist wirkungslos. Legt man unbefruchtete Eier von Arbacia zwei Stunden vor der Befruchtung in 6 ccm Seewasser + 5 Tropfen Limulusserum, so findet in vielen Fällen nur eine sehr geringe Entwicklung der so behandelten Eier statt, oder die Entwicklung bleibt sogar ganz aus. Doch findet zuweilen auch eine Entwicklung solcher Eier statt. Hummerserum und Magendarmsaft von Libinia und anderen Crustaceen haben diese Wirkung nicht. Eier, die zwei Stunden lang vor der Befruchtung in 6 ccm Seewasser + 5 Tropfen Magendarmsaft eines Krebses gelegen hatten, entwickelten sich später häufig in der Weise, daß die einzelnen Blastomesen sich ganz oder teilweise voneinander loslösen. In allen diesen Versuchen wurde, nachdem die Eier die gewünschte Zeit in der Lösung gelegen hatten, das Seewasser gewechselt und dann die



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Befruchtung vorgenommen. Fügt man das Limulusserum oder den Darmsaft erst nach stattgefundener Befruchtung zu, nachdem also die Membran bereits gebildet ist, so ist keine deutliche Wirkung nachweisbar. Fügen wir eine kleine Menge Limulusserum zu Ctenophoren, deren Bewegung aufgehört hatte, so wird dadurch wieder eine lebhafte Tätigkeit der Cilien angeregt. Der Magendarmsaft hat keine derartige erregende Wirkung. Die Ctenophoren bleiben in diesem bewegungslos. Nachträglicher Zusatz von einigen Tropfen Limulusserum hat aber auch in diesem Falle eine stark erregende Wirkung. Wir sehen also hier eine Wirkungsweise des Blutserums von Limulus, die es nahe legt, in dem Plasma wenigstens eine der Ursachen zu suchen, welche die Agglutination der Blutkörperchen, die in dem Blute, sobald es den Körper verlassen hat, stattfindet, veranlassen könnte. Wir sahen aber auch anderweits, daß die Zellsubstanz allein bereits genügt, um die Agglutination zu bewirken. In all den letzt angeführten Versuchen fanden wir, daß die Wirkung des Hummerserums schwächer ist als die des Limulusserums. Dieser Umstand wäre vielleicht in Erwägung zu ziehen, wenn wir die Verschiedenheit in der Gerinnung des Blutes von Limulus und Hummer erklären wollen. Doch genügen diese Befunde noch keineswegs zu einer befriedigenden Erklärung. Vermutlich beruht die agglutinierende Wirkung des Limulusserums auf seinem Gehalt an einer klebrigen Substanz (Fibrin in Lösung?), welche die sich in dem Serum bewegenden Organismen einhüllt. Darauf beruhen wahrscheinlich auch mehrere andere der oben erwähnten Wirkungen des Serums. Darauf deutet auch die Unwirksamkeit oft filtrierten Serums hin. J. Dewitz[1]) zeigte, daß Froschspermatozoen in Wasser, dem etwas Gummi arabicum zugesetzt wurde, in ähnlicher Weise agglutinierten. Doch mögen vielleicht auch noch andere Faktoren an dieser Wirkung des Limulusserums sich beteiligen.

Eine eingehende Besprechung der Wirkungsweise der gerinnungshemmenden Mittel soll hier nicht gegeben werden, da hierzu noch weitere Versuche nötig sind. Nur einzelne Punkte mögen erwähnt werden.

[1]) Centralblatt für Physiologie 1902.

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Gelatinelösungen könnten vielleicht dadurch wirken, daß sie die Blutzellen und das aus Zellen ausfließende Protoplasma an ihrer Vereinigung hinderten, und auch vielleicht dadurch, daß das Fibrinferment an seiner Ausbreitung gehindert wird. Wärme und ebensowohl Formalin wirken wahrscheinlich hemmend auf das Fibrinferment ein (eine solche Wirkung der Wärme konnte bei der zweiten Gerinnung des Hummerplasmas nachgewiesen werden), es werden wahrscheinlich gleichzeitig auch die Eigenschaften des Protoplasmas der Zellen derart beinflußt, daß dasselbe an Labilität verliert, infolge dessen das Ferment die Zellen nicht so leicht verlassen kann und das Zellprotoplasma weniger leicht zu Fäden ausgezogen werden kann. Die gerinnungshemmende Wirkung der Salze beruht nach Sabbatani[1]) darauf, daß durch einige Salze Calcium präzipitiert wird, und daß die Jonisation von Calcium durch Zufügen gewisser anderer Salze verringert wird, daß also allgemein die Menge des aktiven Calciums verringert wird. Die hier berichteten Versuche scheinen diese Hypothese nicht zu stützen, da ja die Oxalate kaum merklich stärker wirken, wie z B. Natriumnitrat, während doch die Oxalate einen viel stärkeren Einfluß auf Calcium haben. Aber auch eine Beziehung zwischen dem durch die Salzlösung bewirkten Präzipitat und der gerinnungshemmenden Wirkung läßt sich nicht nachweisen. Vielleicht wirken auch die Salzlösungen auf das Zellprotoplasma (bezw. auf das darin enthaltene Ferment), indem sie es durch Wasserentziehung weniger labil machen, ohne es jedoch zu töten; da ja bei der nachträglichen Verdünnung der Salzlösungen durch Wasser die gewöhnlichen Veränderungen an den Zellen vor sich gehen können. Meist finden wir eine Parallelität zwischen der präservierenden Wirkung auf die Zellen und der gerinnungshemmenden Wirkung der verschiedenen angewandten chemischen und physikalischen Agentien. Während die zweite Gerinnung des Hummers den Einfluß des Calciums sehr deutlich erkennen läßt, ist dies bei der ersten Koagulation nicht der Fall. Hier sind viel stärkere Eingriffe nötig, um die Gerinnung zu beeinflussen. Dies mag entweder darauf beruhen, daß wie oben ausgeführt, die Agglutination eine

[1]) L. Sabbatani. Comptes rendus de la société de Biologie. 1902. Ref. nach chem. Centralblatt 1902 No. 21.

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wichtige Rolle bei der ersten Gerinnung spielt, oder vielleicht auch darauf, daß bei der ersten Gerinnung jede Zelle ein Gerinnungscentrum bildet, so daß hier gleich im Beginn eine in allen Teilen des Blutes zur Geltung kommende Fermentwirkung vorliegt. Die Bedeutung des von der ersten Koagulation stammenden fädigen Gerinnsels für die zweite Gerinnung des Hummerplasmas, sowie der Umstand, daß die Zellen den Hauptbestandteil des fädigen Gerinnsels bilden und daß dieselbe durch die bei dem Wirbeltierblut zur Defibrination benutzte Methode von dem Fibrin nicht zu trennen sind, legen den Gedanken nahe, daß doch ein wenn auch vielleicht nur quantitativer Unterschied in den Beziehungen der Blutzellen der Arthropoden einerseits und der Vögel andererseits zur Blutgerinnung bestehen muß.

Die Bedeutung der Blutkörperchen für die Gerinnung des Blutes besteht also bei Arthropoden 1. darin, daß das ausgeflossene Zellprotoplasma und die agglutinierten Zellen eine fädige Substanz liefern, die in physikalischer Hinsicht vom Fibrin kaum unterschieden werden kann; 2. darin, daß die Blutzellen eine Substanz enthalten, die bewirkt, daß aus dem Blutplasma sich Fibrin um die Blutzellen abscheidet. Eine gewisse, aber in diesem Falle nicht eindeutige Beziehung zwischen den Blutzellen und der Gerinnung zeigt sich auch darin, daß in dem Blute eines sterbenden Limulus, der, wie wir früher erwähnten, nur wenig Blutkörperchen besitzt, sich auch nur eine geringe Menge Fibrin bildet.

Über die Bedeutung der Blutkörperchen bei Limulus für die Entzündung.

Nachdem wir das Verhalten der Blutkörperchen bei der Gerinnung besprochen haben, können wir uns über das Verhalten derselben bei gewissen Schädigungen im Innern des Tierkörpers kurz fassen. Führen wir, wie das in einer früheren Arbeit[1]) beschrieben wurde, Fremdkörper wie Agar oder geronnenes Eiweiß oder einen Faden in das Tier ein, so verhalten sich die Blutzellen von Limulus wesentlich anders wie die Leukocyten eines Säugetieres. In letzterem Fall wandern schon nach

[1]) On the bloodlymphcells etc. The Journal of Medical Research. Vol. II, 1902.

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7 Stunden etwa die mehrkernigen Leukocyten in den Agar ein und sie bilden bei ihrem Vordringen baumartige Verzweigungen in dem Agar. Zellen anderer Art bilden später fibrilläres Bindegewebe und zuweilen auch Riesenzellen um den Agar; diese Riesenzellen wiederum gehen später zu Grunde. Gleichzeitig hiermit dringt das Bindegewebe in den Agar ein und umgibt seine Außenseite mit einer Kapsel. Bei Limulus verhalten sich die Körperzellen Fremdkörpern gegenüber anders. Es wurde die Einwirkung von Fremdkörpern bis zu 15 Tagen nach ihrer Einführung verfolgt, und es wurden Stücke in verschiedenen Zeiträumen mikroskopisch untersucht. Es ließ sich feststellen, 1. daß die Blutkörperchen niemals in den Agar oder das Eiweiß eindringen. Wohl fanden sich zuweilen Blutzellen in Spalten am Rande des Agar. Aber in diesem Falle handelte es sich allem Anschein nach um durch mechanisches Zerreißen produzierte Spalten, in welche die Blutkörperchen mechanisch hineingerieten. Die Bilder waren durchaus verschieden von denen, welche nach Einführung von Fremdkörpern beim Meerschweinchen erhalten werden. Hingegen bilden die um den Agar oder um Fäden angesammelten Blutzellen ein ähnliches Gerinnsel, wie dies im Blute außerhalb des Körpers geschieht. Das Blut mit samt den Zellen umgibt den Fremdkörper und dringt in die vorgebildeten Spalten ein. Hierbei zeigen die Blutkörperchen und das Blut dieselben Veränderungen, die auch außerhalb des Körpers stattfinden, es findet Blutgerinnung und wahrscheinlich auch eine Agglutination der Blutzellen statt. Allmählich wird diese geronnene Masse nekrotisch, ihre Kerne schwinden. Zuletzt ist der Fremdkörper von einer konzentrisch gelegenen homogenen, hyalinen Masse, welche keine weitere Struktur zeigt, umgeben. Doch soll hervorgehoben werden, daß man nicht selten einzelne Muskeln eines Limulus, in den Fremdkörper eingeführt waren, teilweise aufgelöst findet und daß in den Buchten solcher Muskeln Zellen liegen, die den Blutkörperchen ähnlich sind und die sehr den Osteoklasten der Knochen gleichen. Vielleicht haben diese Zellen eine phagocytische Funktion. Es ist nicht gelungen, Mitosen in dem die Wunde umgebenden Gewebe zu finden. Die Einführung eines Fremdkörpers in einen Limulus bewirkt Ver-

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änderungen, welche am ehesten mit der Thrombose zu vergleichen ist, die nach Verletzung der Gefäßwand in dem Blutgefäß eines Säugetieres stattfindet. Es besteht in mancher Hinsicht eine Ähnlichkeit in dem Verhalten der Blutplättchen der Säugetiere und der Blutkörperchen oder Teile der Blutkörperchen von Arthropoden.

Es soll nun noch kurz erörtert werden, ob mehrere Arten von Blutkörperchen in diesen Arthropoden vorliegen. Bei Limulus habe ich durch Beobachtung der Blutkörperchen in dem Tiere selbst und durch Färbung derselben nach dem Ausfließen des Blutes keinen Hinweis darauf erhalten können, daß hier mehrere Arten von Blutkörperchen vorhanden sind. In Bezug auf die Blutkörperchen des Hummers wurde schon oben darauf hingewiesen, wie schwer es ist, die Blutkörperchen außerhalb des Körpers vor Veränderungen zu bewahren. Gewisse Verschiedenheiten zwischen Blutzellen, auf die eine Einteilung der normalen Blutkörperchen des Hummers begründet wurde, beruhen auf Auflösungsprozessen, welchen die Zellen nach dem Verlassen des Körpers unterliegen. Ich konnte mich nicht überzeugen, daß beim Hummer granulalose Blutkörperchen normal vorkommen. Ob Unterschiede geringeren Grades zwischen verschiedenen Blutkörperchen des Hummers in Bezug auf die Kernform oder Färbung der Granula bestehen, darüber habe ich keine besonderen Untersuchungen angestellt. Solche Untersuchungen dürften wegen der angegebenen Fehlerquellen sehr schwer sein. Die Größe der Granula, die Form des Kernes verändert sich in vielen Blutkörperchen, sobald sie sich unter experimentellen Bedingungen befinden. Nur soviel konnte festgestellt werden, und das scheint wesentlich zu sein, daß nämlich in physiologischer Hinsicht, in ihrem Verhalten während der Gerinnung, in ihrer Reaktion auf mechanische Einflüsse, Fremdkörpern gegenüber irgend welche durchgreifenden Unterschiede zwischen den einzelnen Blutkörperchen nicht beobachtet werden konnten.

Über gewisse Analogien zwischen dem Verhalten der Blutzellen der Arthropoden und gewisser Zellen von Wirbeltieren.

Nachdem wir uns nun so weit in der Hauptsache auf die Darstellung von Beobachtungen und von Versuchen und der nächst-

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liegenden, daraus zu ziehenden Schlußfolgerungen beschränkt haben, soll auf einige Analogien hingewiesen werden, die zwischen einzelnen hier beschriebenen und gewissen anderen bei höheren Tieren beobachteten Vorgängen bestehen. Solche Analogien müssen bei der Analyse dieser letzteren in Berücksichtigung gezogen werden.

Bei Schädigungen von Geweben im Wirbeltierkörper findet eine Auswanderung der weißen Blutkörperchen aus den Blutgefäßen nahe dem verletzten Bezirk in diesen hinein statt. Diese Wanderung wird nun gewöhnlich als eine Äußerung der positiven Chemotaxis der Leukocyten aufgefaßt. Das Verhalten der Blutkörperchen der Arthropoden während der Koagulation des Blutes macht es nun sehr wahrscheinlich, daß diese Leukocytenwanderung nicht lediglich durch Chemotaxis bewirkt wird. Außerhalb des Körpers befinden sich die Blutkörperchen der Arthropoden unter Bedingungen, die offenbar eine gewisse Analogie darbieten, zu den Verhältnissen, in denen sich die Leukocyten höherer Tiere bei Gewebsschädigungen befinden. Bei der Wanderung und Ausbreitung der Arthropodenzellen sind nun wahrscheinlich keine bestimmt gerichteten chemischen Reize vorhanden. Diese Zellen befinden sich anscheinend in einem homogenen Medium außerhalb des Körpers. Die Ausbreitungserscheinung der Blutkörperchen beruht auf einer Veränderung des Zellprotoplasmas, die durch unbekannte chemische oder physikalisch-chemische Agentien hervorgerufen wird, aber ohne daß ein bestimmter Richtungsreiz vorliegt. Wie bei den Blutkörperchen der Arthropoden dürfte es sich bei chemotaktischen Erscheinungen wohl um Veränderungen der Oberflächenspannung der Zellen handeln. Bei den Blutzellen der Arthropoden finden gleichzeitig mit einer solchen Änderung der Oberflächenspannung noch Auflösungserscheinungen in dem Protoplasma statt. Also Bewegungen und Ausbreitungserscheinungen der Blutkörperchen finden statt, wenn dieselben in einem gleichmäßigen Medium liegen, welches Veränderungen in dem Protoplasma hervorruft, ohne daß gewisse chemische Stoffe aus bestimmter Richtung die Zelle treffen müssen, und ohne, daß die Konzentration dieser chemischen Substanzen in einer bestimmten Richtung an Stärke zunehmen muß. Die Ähnlichkeit der Ausbreitungsprozesse der

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körperchen der Säugetiere auf Entzündungsreize ist so groß, daß wir wohl annehmen können, daß auch hier die Auswanderung ohne eine direkte chemisch richtende Ursache stattfinden kann und primär bedingt ist durch in dem Zellprotoplasma hervorgerufene Veränderungen, die auf allen Seiten durch die umgebende Flüssigkeit in den Zellen veranlaßt wird. Hiezu treten dann wahrscheinlich chemotaktische Reaktionen als ein weiterer Faktor hinzu.[1])

b) Schon oben wurde darauf hingewiesen, daß eine deutliche Ausbreitung der Blutzellen nur in Berührung mit festen Körpern, wie sie z. B. die Fläche des Objektträgers darstellt, beobachtet werden konnte. Die Blutkörper kleben in Berührung mit solchen Flächen fest. Das Zellprotoplasma wird durch Erweichungsprozesse (wenigstens an der Seite der Berührung mit dem festen Körper) in eine klebrige Substanz verwandelt. Dieser Umstand allein genügt in diesem Falle, um ein Wandern in Berührung mit festen Körpern zu veranlassen. Auch das regenerierende Epithel bewegt sich in Berührung mit festen Körpern, und ich konnte beobachten, daß das epitheliale Protoplasma fest an dem Schorf haftet und nicht selten beim Abreißen des Schorfes zum Teil mit ihm entfernt wird. Dies legt die Annahme nahe, daß beim regenerierenden Epithel vielleicht ähnliche, wenn auch nicht so starke Veränderungen des Protoplasmas, wie an den Blutkörpern vor sich gehen, die das Haften des Epithels an festen Körpern veranlassen.

c) Innerhalb des Körpers werden die ovalen Blutkörperchen, ohne Fortsätze auszusenden und ohne ihre Gestalt zu ändern, im Blut durch die Bewegung der Flüssigkeit umher getrieben. Sobald das Blut den Körper verläßt, treten Veränderungen in

[1]) Es muß allerdings die Möglichkeit berücksichtigt werden, daß doch sehr geringfügige Unterschiede in der Stärke bestehen, in der die Einwirkung des umgebenden Mediums die einzelnen Blutkörperchen auf verschiedenen Seiten beeinflußt. Dann tritt in der das Protoplasma als ganzes verändernden Wirkung des umgebenden Mediums doch noch eine bestimmt richtende Wirkung hinzu. Jedenfalls findet neben der chemotaktischen Beeinflussung eine allgemeinere Veränderung des Protoplasmas der Blutzellen, der Arthropoden unter der Entzündung ähnlichen Bedingungen statt.


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dem auf die Zelle einwirkenden Bedingungskomplex ein und die Zellen schließen sich zu einem großen Teil zu einer Masse zusammen, die mit einem Gewebe verglichen werden kann. Diese Tatsache würde nicht dadurch umgestoßen, daß etwa extracellulär entstehendes Fibrin den Zellen beigemischt sein sollte. Die Zellen selbst bleiben hierbei nicht unverändert, sie geben einen Teil ihres Protoplasmas nach außen ab; sie bilden eine Zone von Exoplasma, welches scheinbar die eigentliche Zelle mit Kern und Granula einschließt. Aus dem außerhalb des endoplasmatischen Teiles der Zelle, also desjenigen Teiles, der jetzt als die eigentlichen Zellen erscheint, bilden sich unter dem Einfluß geringfügiger Bewegungen Fasern. Auch ganze Zellen können zu Fasern umgewandelt werden und zwischen solchen Fasern bleiben dann Zellreste mit Kern und relativ wenig Protoplasma übrig. Wir haben es hier mit der Bildung einer intercellulären Substanz auf Kosten von Zellprotoplasma zu tun. Dieser Prozess bietet Vergleichspunkte mit der Entstehung von Bindegewebe aus Bindegewebszellen. Machen wir Schnitte durch geronnenes Blut vom Limulus eine Stunde nach der Entnahme des Blutes, so sehen wir bei Färbung nach Mallory schön blau gefärbte Fasern anstelle des Exoplasmas. Ganz ähnliche Vorgänge scheinen nach Mall[1]) bei der Bildung des Bindegewebes stattzufinden. In dem frischen, nicht fixierten Blutkoagulum sehen wir deutlich, daß die Zellen von einem homogenen Exoplasma umgeben waren. Auch hier konnten wir leicht durch geringe Bewegungen Fasern produzieren, die allerdings nicht auf das Exoplasma beschränkt sein müssen. Die bei der mikroskopischen Bearbeitung des Materials stattfindenden mechanischen Zugwirkungen genügen offenbar, um diese Fibrillation und zwar hauptsächlich in dem Exoplasma zu bewirken. Auch in den Fortsätzen des Zellprotoplasmas selbst können Fasern gebildet werden, wie das ja wohl auch bei der Bildung des retikulären Bindegewebes geschieht. Nach Vollendung der hyalinen Ausbreitung der Zellen gleicht das von den Blutzellen gebildete

[1]) Mall, American Journal of Anatomy 1902. In ähnlicher Weise, nämlich hauptsächlich aus dem exoplasmatischen Teile eines retikulären Syncytimus scheint sich nach Hardesty auch die Neuroglia zu bilden. (American Journal of Anatomy.) 1902.

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Gewebe durchaus dem myxoiden (retikulären) Bindegewebe. Nun bestehen ja sicher Verschiedenheiten zwischen einem solchen Gewebe und dem Bindegewebe. Chemisch sind beide wahrscheinlich verschieden. Auch morphologisch besteht keine absolute Identität. Aber die Ähnlichkeit in einzelnen wesentlichen Punkten ist doch so groß, dass wohl die Bildung dieses Blutzellengewebes als eine „akute" Bildung eines Bindegewebes bezeichnet werden kann. Amöboide Bewegungen der Zellen, wie sie hier beobachtet werden, dürften auch bei der Bindegewebsbildung stattfinden. Auch bei dieser dürfte den mechanischen Umständen (Zug) eine Bedeutung für die Fibrillation des cellulären Protoplasmas und besonders für die Richtung der Fibrillen zukommen. Natürlich ist bei beiden Zellarten neben den äußeren mechanischen Faktoren wie Zug die Konstitution des Protoplasmas ein wesentlicher Faktor, ohne den der Zug die Fibrillenbildung nicht bewirken könnte. Vielleicht können wir aber aus den bei der Bildung des Blutzellengewebes gemachten Beobachtungen einige weitere Schlüsse auf die bei der Bindegewebsbildung direkt kaum zu beobachtenden Vorgänge ziehen.

Wir sahen, daß ein Teil des Protoplasmas der Zellen ausfließt und daß aus diesem nun völlig extracellulär gelegenen Protoplasma ebenfalls Fibrillen gebildet werden können. Sollte nicht auch etwas ähnliches bei der Bindegewebsbildung stattfinden? Es würde dann die von Kölliker, Merkel und anderen angenommene Bildung von extracellulären Fibrillen auch vorkommen; diese entständen aus ausgeflossenem Zellprotoplasma. Bei der Bildung von Knorpel hat Spuler[1]) das Vorkommen von intercellular gelegenen Granula beschrieben. Bei dem Blutzellengewebe können wir deutlich verfolgen, wie Granula in den intercellulären Teil des Gewebes geraten, dadurch, daß ein Teil des Protoplasmas ausfließt, oder auch, indem ganze Zellen zerfließen, oder endlich dadurch, daß im Laufe der amöboiden Ausbreitung Protoplasmateile mit Granula sich so weit von dem um den Kern gelegenen Protoplasma der Zellen trennen, daß sie nicht mehr zu der Zelle zu gehören scheinen. Auch werden solche Granula in Fasern, welche aus dem Zellprotoplasma ent-

[1]) Spuler, Zieglers Beiträge 1902.

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stehen, eingebettet. Eine weitere Möglichkeit, die zu erwägen wäre, ist die folgende: Veränderungen in dem Medium, in dem die Blutzellen sich befinden, verursacht die Vorgänge, die zu der Bildung von Intercellularsubstanz aus Zellen führen. Der Prozeß kann vermieden werden durch chemische oder chemisch-physikalische Änderung des umgebenden Mediums, z. B. durch Auffangen des Blutes in gesättigten Salzlösungen. Also ein wesentlicher Faktor, der die Bildung der intercellulären Fibrillarsubstanz bedingt, ist die Beschaffenheit der Flüssigkeit, in der sich die Zellen befinden. Diese Tatsache kann auch in folgender Weise ausgedrückt werden. Das Serum, in dem die Zellen sich außerhalb des Körpers befinden, ist für die Blutkörperchen cytolytisch. Die Bildung einer fibrillären Intercellularsubstanz beruht auf der Wirkung einer die Zellen nicht mehr vor dem Zerfall bewahrenden, also cytolytischen Flüssigkeit.

Durch chemische Änderung, z. B. bei Verwendung von saturierten Salzlösungen, kann diese cytolytische Flüssigkeit in eine zellerhaltende umgewandelt werden. Dies schließt nicht die Möglichkeit aus, daß die cytolytische Wirkung des Serums möglicherweise nur eine indirekte ist, indem das Serum als ein Medium dient, welches die durch die Anwesenheit anderer Körper bedingten Veränderungen den Zellen zuleitet. Diese Erwägungen leiten zu einer ähnlichen Hypothese in Bezug auf die Bildung des fibrillären Bindegewebes. Sollte nicht auch im Tierkörper eine der Bedingungen der Bindegewebsbildung aus Bindegewebszellen in einer relativen, cytolytischen Tätigkeit der Gewebsflüssigkeit oder der durch die Gewebsflüssigkeit vermittelten Veränderungen bestehen? Und sollte nicht auch hier eine andere Zusammensetzung der Gewebsflüssigkeit die Zellen vor dem teilweisen Zerfall bewahren und dadurch die Bildung von Intercellularsubstanz und Narbengewebe verhindern können? Im Körper ist wohl immer die zur Fibrillation nötige mechanische Zugwirkung infolge der Bewegungen des Tieres vorhanden. Auch die aus Bindegewebszellen sich bildenden Fasern retrahieren sich ebenso wie die aus den Blutzellen gebildeten.

Wir sahen, daß mechanische Momente auf die Gestaltung dieses Blutzellengewebes einen wesentlichen Einfluß haben. Zug bewirkt fibrilläre Umwandlung des Protoplasmas, sowohl des

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Protoplasmas im Inneren einer Zelle. Die fibrilläre Struktur des Protoplasmas ist also eine sekundäre, durch Zugwirkungen bedingte. Aber nicht nur die Entstehung der Fibrillen infolge von Zugwirkungen kann in diesem Falle beobachtet werden, sondern auch die Richtung der gebildeten Fasern, wie das schon oben erwähnt wurde. Sehr deutlich ist meist nachzuweisen, daß mechanische Momente komplizierte fibrilläre Strukturen hervorbringen, wenn wir z. B. einen Tropfen Limulus- oder Hummerblut auf einen Objektträger auffallen lassen. Nach kurzer Zeit sehen wir dann bei eintretender Gerinnung ein System konzentrischer Kreise entstehen, welche durch radiäre Züge verbunden sind, als eine Folge der vom Centrum nach außen in dem auffallenden Tropfen sich fortpflanzenden Wellenbewegung. In diesem Fall nahmen nun auch extracellular entstehende Fasern an der Bildung dieser Strukturen teil. Aber wir wissen, daß die Zellen unter diesen Umständen ganz in derselben Weise durch geringfügige mechanische Einflüsse angeordnet und zu Fasern umgewandelt werden. Auch im Knochen werden ähnliche komplizierte Faserstrukturen durch Zug und Druckwirkungen veranlaßt.

Wir wiesen bereits oben darauf hin, daß sehr oft aus den Blutzellen entstandene Fibrillen sich ununterbrochen durch das Gebiet mehrerer Zellen kontinuierlich fortsetzen. Kontinuierliche Fibrillen finden wir auch in anderen Geweben. Die auf gewissen Stadien der Regeneration in Epithelzellen sehr deutlich werdenden Protoplasmafasern ziehen ebenfalls durch mehrere Zellen hindurch. Ich habe in einer früheren Arbeit[1]) darauf hingewiesen, daß diese epithelialen Fibrillen dem Zug entsprechend angeordnet sind, den die vordringenden Epithelzellen ausüben. Wir sehen hier also wiederum wie ein mechanisches Moment die Richtung und vielleicht auch die Entstehung der Fibrillen bestimmt. Die Fibrillen des regenerierenden Epithels sind nicht die Fibrillen der ruhenden Epithelzellen, sondern müssen neu gebildet sein. Fibrillen, die kontinuierlich durch mehrere Zellgebiete hinziehen, liegen auch bei der Herzmuskulatur und nach neueren Untersuchungen vielleicht auch im peripheren Nerv vor.

[1]) Über die Regeneration des Epithels, Arch. f. Entwicklungsmechanik. 1898.


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Obwohl natürlich in allen diesen Fällen die Konstitution der verschiedenen Zellarten der wesentliche Faktor für die fibrilläre Struktur dieser Gewebe ist, dürfte mechanischen Umständen vielleicht auch hier eine gewisse Bedeutung als auslösenden Faktoren zukommen.

Wir sahen, daß die Blutzellen sich in gewissen Flüssigkeiten in epithelialer Form anordnen. Dies beruht auf einfacher Agglutination von Zellen, die besser erhalten bleiben, als dies im Blutserum gewöhnlich der Fall ist.

Es wurde auf diese Analogien hingewiesen, nicht in der Annahme, daß eine vollständige Identität der zum Vergleich herbeigezogenen Vorgänge mit den bei den Blutzellen beobachteten vorliege; wohl aber ist es nicht unwahrscheinlich, daß gewisse wesentliche Faktoren bei beiden Reihen von Vorgängen identisch sind, und deshalb dürfte ein der experimentellen Prüfung leicht zugängliches Objekt, wie die Blutzellen, bei der Analyse der bei der Entzündung und Gewebsbildung stattfindenden Vorgängen, die der experimentellen Untersuchung nur schwer zugänglich sind, nicht ohne Wert sein.

Einige der Ergebnisse dieser Untersuchung mögen zum Schlusse zusammengefaßt werden:

1. Bei der Gerinnung des Blutes der Arthropoden kommen folgende Faktoren in Betracht: a) Agglutination der Blutzellen; b) Vereinigung des aus Zellen ausgeflossenen Protoplasmas und des Protoplasmas vollständig zerflossener Zellen zu einer gelatinösen Masse und sekundär zu Fäden; c) die in der nächsten Stunde stattfindende Ausbreitung der Blutzellen; d) die Gerinnung einer fibrinogenen Substanz.

2. Das Zusammenfügen der Blutzellen führt zu gewebsartigen Bildungen. Die Bildung der fibrillären Zwischensubstanz entspricht der Bildung eines fibrillären Bindegewebes. Durch Veränderung der Flüssigkeit, in der die Zellen suspendiert sind, kann der cytolytische Einfluß des die Zellen umgebenden Mediums aufgehoben und die Bildung der fibrillären Zwischensubstanz verhindert werden. Durch Agglutination von Zellen, welche erhalten bleiben, kann eine epitheliale Anordnung der Zellen entstehen.

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selben in verschiedenen Lösungen verhindert oder beschränkt werden. Meistens haben diese Lösungen auch einen erhaltenden Einfluß auf die Zellen. Oxalate wirken ähnlich wie andere Salzlösungen, nämlich nur in saturierter bis halbsaturierter Lösung. Das weist darauf hin, dass wahrscheinlich eine Einwirkung auf Calcium bei dieser Gerinnungshemmung nicht der wesentliche Faktor ist. Natrium und Kaliumsulfat haben ebenso wie Natriumnitrat und Natrium und Kaliumchlorid einen gerinnungshemmenden Einfluß, aber die Blutzellen werden verschieden beinflußt von den deiden Sulfaten einerseits und den anderen Salzen andererseits.

4. Die Gerinnung des Blutes wird gehemmt durch Auffangen des Blutes in Gelatinelösungen. Auffangen in Öl ist ohne Einfluß.

5. Die zweite Gerinnung des Hummerblutes wird durch andere Mittel verhindert, wie die erste Gerinnung. Die bei der zweiten Gerinnung beobachteten Tatsachen können erklärt werden durch die Annahme der Tätigkeit eines Gerinnungsfermentes, welches in den Blutzellen und dem Muskel des Hummers, nicht aber in den Blutzellen oder dem Muskel gewisser Wirbeltiere verbunden ist. Dies weist auf eine Vielheit der Fibrinfermente in verschiedenen Tierarten und auf eine Identität der Fermente in verschiedenen Geweben derselben Tierart hin. Die Spezifität in dem einen Fall, bezw. der Mangel an Spezifität in dem andern Fall entsprechen einer ähnlichen Spezifität, bezw. einem Mangel an Spezifität, wie sie für Präzipitine, die durch Injektion von Körpersäften oder Eiweißstoffen erhalten worden, festgestellt wurden.

6. Kaliumcyanid, Harnstoff, Peptonlösungen hemmen die zweite Gerinnung. Hierbei ist die Stärke der hemmenden Einwirkung dieser Stoffe bei Mischung mit dem Plasma gerade die umgekehrte, wie wenn sie auf das Ferment enthaltende Fibrin wirken. Kaliumcyanid wirkt am stärksten auf das Fibrin, Pepton am schwächsten, umgekehrt wirkt Pepton am stärksten, Kaliumcyanid am schwächsten bei Vermischung mit dem Plasma.

7. Die Notwendigkeit der Anwesenheit des Calcium läßt sich für die zweite Gerinnung leicht nachweisen. Ammonium-


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chlorid hemmt die zweite Gerinnung stärker wie Natriumchlorid.

8. Zug und Druckwirkungen verwandeln das Zellprotoplasma in ein Fasersystem, das von dem extracellulären Fibrin in seinem Aussehen und in seinen physikalischen Eigenschaften nicht zu unterscheiden ist. Die Zellgranula verschwinden unter dem Einfluß der Zug- und Druckwirkung, ebenso wie dies spontan in den Zellen in dem Blutserum außerhalb des Körpers stattfindet.

9. Durch Zug- und Druckwirkung können in dem zellfreien Blutserum ähnliche fibrilläre Strukturen hervorgerufen werden, wie aus dem Zellprotoplasma. Der kolloidalen Lösungen gewisser Eiweißstoffe und dem Zellprotoplasma in gleicher Weise zukommende chemische Charakter kommt für einen Teil der morphologischen Charaktere der Zellen allein in Betracht, insbesondere für die unter gewissen mechanischen Bedingungen auftretenden fibrilläre Struktur gewisser Zellen (Zellenteile) oder Gewebe.

10. Zug- und Druckwirkungen bestimmen auch die Richtung der aus Zellen entstehenden fibrillären Strukturen.

11. Die Bedeutung der Blutzellen für die Gerinnung besteht darin, daß sie 1) sich selbst in eine fibrinähnliche Masse umwandeln, und daß sie 2) die Gerinnung in dem umgebenden Blutplasma hervorrufen, wobei einzelne Tatsachen auf die Möglichkeit hindeuten, daß die im Plasma enthaltene gerinnende Substanz aus den Blutzellen stammt.

12. In den Körper eingefügten Fremdkörpern (Agar) gegenüber verhalten sich die Blutkörperchen von Limulus nicht so aktiv wie Säugetierblutkörperchen. Ein Eindringen in dieselben wird nicht beachtet. Sie beteiligen sich an der Bildung eines Gerinnsels um den Fremdkörper. Aktive Proliferation anderer Zellen oder Riesenzellenbildung wurde um die Fremdkörper nicht beobachtet.

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Notes on Technique, III.

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NOTES ON TECHNIQUE, III.

On Injecting the Blood Vessels of the Earthworm.

In specimens of the earthworm prepared for dissection by preservation in alcohol, with or without previous treatment with chromic acid, the blood vessels are always found to be contracted and shrunken and not showing the characteristic color of the blood except in the largest vessels. Unless then an abundance of live worms are at hand which can be used for the study of the vascular system, class work on this particular subject is apt to be unsatisfactory. It is not usually convenient to furnish living material in quantity for the study of the circulatory system alone, and it has seemed highly desirable to make if possible injected preparations of the blood vessels so that students would be able to make out points which their own specimens did not show. After some experimenting on the subject it was found that good injections of the blood vessels of the common earthworm could be obtained. As the operation is in practice a rather difficult one, and since in the experience of the author it is impossible to attain successful results by the use of the ordinary injection methods, it seems advisable to publish an account of the procedure employed.



FIG. 1.—Showing form of the glass tube used for injection in place of syringe. About one-half natural size.

The main difficulties encountered were of two different sorts: (a) the smallness of the vessels made the introduction of the cannula very difficult, and (b) the walls of the vessels were so thin that they would withstand only the gentlest and steadiest of pressure. All attempts to inject by means of a syringe (hypodermic or larger) were unsuccessful. Furthermore, none of the masses ordinarily used for macroscopic injection nor any of several of the masses advocated for microscopic work were found to be adapted to this work. Either the mass would not run in the vessels or else it would cause them to burst, or finally it would not "set" after filling the vessels.

It was finally found best to use a bent glass tube drawn to a fine point at one end (Fig. 1) in place of a syringe. By blowing at the upper end of such a tube partially filled with injection mass it was found that a much steadier and more easily regulated pressure could be obtained than with any syringe. Furthermore, the tube could be better inserted into the vessels and held more steadily after

1 Tandler, J. Mikroskopische Injectionen mit kaltflüssiger Gelatin. Zeitschr. f. wiss. Mik. **18**: 22–24, 1901. Reviewed in this journal, **5**: 1625 and 1626.

It was found necessary to use a slightly larger amount of KI than is recommended by Tandler in order to keep the gelatine fluid until after injection. Eight to ten grams of the KI to 100 c. c. of fluid were used.

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insertion than an ordinary cannula attached to a syringe. The injection which was found to give the best results was the cold gelatine mass recently devised by Tandler[1] for microscopic work. The mass was colored with a small amount of a saturated aqueous solution of anilin blue-black.

In detail the procedure was as follows: Large specimens of the common earthworm (*Lumbricus agricola*) were stupified in the ordinary way by the use of alcohol. They were not allowed to become entirely stupified, but just as soon as they had become sufficiently quiet they were opened with fine scissors along the dorsal side just to one side of the mid-dorsal line. The cut began just behind the clitellum and was carried forward in some cases clear to the anterior end, in other cases only to a point about two centimeters in front of the clitellum. After making this first cut the septa were cut down close to the body wall for a short distance only and the specimen stretched lengthwise and pinned out in a wax-bottomed dissecting pan in normal salt solution (.75 per cent. NaCl). No attempt should be made to dissect the septa down far enough to admit of pinning the specimen out flat, as it is almost impossible to avoid cutting some of the larger vessels. It was only found necessary to spread the worm enough so that the vessels on the intestine could be seen.

The glass tube was partially filled by suction with the injection mass, and its pointed end was inserted into the dorsal vessel, the point being directed forwards. It was found best to introduce the tube into the vessel at a point just under the clitellum, as here the dorsal vessel is considerably larger than at any other point in its course. It is necessary to get the tube into the vessel very soon after the worm is opened or it will not be possible to insert it at all, as the vessels contract strongly soon after they are exposed. It is not necessary to make any cut in the vessel for the insertion of the tube as the wall is so thin as to admit of its being pushed in directly. No ligature was used. After the tube is inserted, a steady, even pressure may be applied to the upper end of the tube by blowing, and in this way the mass may be started into the vessels. It was necessary to use a very gentle pressure at first, or otherwise the vessel would break immediately in front of the point of the tube. When once started the mass will usually run very freely and the pressure may be increased without rupturing the vessels. The injection should be continued till all visible vessels are distended. The tube may then be removed and the specimen put in 4 per cent. formalin for preservation and to harden the injection mass.

Specimens injected in this way have the dorsal vessel and all the vessels which come off from it in the region in front of the clitellum well filled. The parietal, intestinal and lateral œsophageal vessels fill completely and the excretory plexus shows very well. The "hearts" present a very striking appearance. For some reason which was not apparent the injection mass could not be made to pass from the "hearts" into the subintestinal vessel. Instead this vessel became distended with blood. No practical difficulty is caused by this lack of injection of the subintestinal vessel, as it is sufficiently large to be traced with ease in any ordinary specimen. By dissecting and pinning the specimens out in glass dishes containing a layer of black wax on the bottom, permanent preparations may be made.

RAYMOND PEARL.

Zoological Laboratory, University of Michigan.

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Notes on Technique, I-II.

RAYMOND PEARL and LEWIS H. WELD.

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NOTES ON TECHNIQUE, I-II.*

I. *The Preparation of Sections of Hydra for Class Work.*

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The preparation of satisfactory sections of Hydra for microscopical study of the tissues has always been attended with more or less difficulty. Usually the cell boundaries are poorly defined, sub-epithelial cells are not clearly marked off, and on account of the presence of large quantities of metabolic products—fat globules, etc.—in the cells, the sections as a whole have an indefinite, crowded appearance. After some experimentation the following method of preparing and staining sections of Hydra was tried with excellent success. Its results are very satisfactory; cell boundaries are clearly marked, so that sub-epithelial cells may readily be seen by the student, the muscle processes appear with entire distinctness, and finally the cytological relations of the cells are well defined.

The method used is in detail as follows: Large specimens of the common brown Hydra (H. fusca) are placed in Stender dishes filled with filtered tap water and allowed to "starve." The purpose of this "starving" process is to rid the cells of stored-up fat and other metabolic products. Since the animals are placed in dishes containing only clear water they are unable to feed and so use up gradually these reserve and intermediate products of assimilation. This "starving" is perhaps the most important step in the process, as on its thoroughness the character of the sections depends. The length of time which must be allowed for the animals to become entirely free of fat globules will depend on their condition at the beginning. We have found in practice that from one to two weeks is usually a sufficient length of time. When the process is completed the animals are very light, in fact almost white, in color and are more than ordinarily transparent when fully extended. Some of the specimens may die in the course of the "starving" process, but not any considerable number are lost in this way.

When thoroughly "starved" the animals are fixed in vom Rath's picro-osmo-aceto platinic chloride mixture[1] for about an hour, then washed in methyl alcohol, blackened for several hours in pyroligneous acid, again washed in methyl alcohol, removed to 70 per cent. alcohol and carried up through 96 per cent. and absolute alcohol, cleared in cedar oil, and embedded in paraffin in the ordinary way.

The animals are killed almost completely extended in the following way. A Hydra isolated in a pipette with a small amount of water is placed on a slide or

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* These "Notes" do not make any claim for originality, but are merely detailed accounts of some applications of ordinary methods which have been found in practice to accomplish in a satisfactory way the desired ends. They are offered in the hope that they may prove useful to teachers. R. P.

1 vom Rath, O. Zur Conservirungstechnik. Anat. Anz. II: 280–288, 1895.
Formula (p. 283):
200 c. cm. saturated aqueous solution of picric acid.
Added in the order given: 25 c. cm. 2 per cent. aqueous solution of osmic acid.
1 g. platinic chloride, dissolved in 10 c. cm. of water.
2 c. cm. glacial acetic acid.
Fixation in Hermann's fluid, with subsequent treatment with pyroligneous acid in the same way, has been found to give equally good results.

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in the bottom of a watch-glass with a drop of water just large enough to cover it. If then placed in fairly strong light, as on the stage of a microscope over the opening of the condenser, the animal will soon expand to its full length. Then from a pipette filled with the vom Rath mixture a stream of this strong fixing agent is suddenly and forcibly thrown on the Hydra. The specimen will, provided the stream is directed so as to strike it squarely and is sent with some force, in nearly every case be killed before it contracts to any extent.



FIG. 1.—Photograph of portion of cross section of Hydra showing character of preparation made by the method described. The photograph is much less clear and precise in definition than the preparation. Along the upper left side of the figure the muscle processes may be distinctly seen just at the inner ends of the ectoderm cells.

Sections are cut five or ten mikrons in thickness, fastened to the slide with Mayer's albumen fixative, and stained by the Heidenhain iron-hæmatoxylin method. The sections are mordanted about thirty minutes in the iron alum (two per cent. solution), *thoroughly* washed in water, stained for thirty minutes in a one-half per cent. aqueous solution of hæmatoxylin, washed again, and differentiated in the iron alum solution. The differentiation should be continued till only the nuclei, cell boundary regions, and muscle processes are stained. No counterstain is used. The sections are then carried up through the different grades of alcohol, cleared in xylol, and mounted in balsam.

The resulting sections are almost diagrammatic in their clearness and are very satisfactory for class use. Such things as the muscle processes, which are ordinarily so difficult of demonstration to students, are so clearly brought out as to cause no trouble whatever. We have appended to this paper a photograph showing, in some degree, the character of the sections prepared in the way just described.

RAYMOND PEARL AND LEWIS H. WELD.

II. *The Demonstration of Nerve Fibers in the Ventral Cord of the Earthworm.*

The following method of making sections of the ventral nerve cord of the earthworm has been found to give preparations which furnish very clear pictures of the structure of nerve cells and their associated axis cylinder processes. The plan was developed with the idea of bringing more forcibly to the student's mind than seems possible with the conventional cross section an adequate conception of the most important structural elements of the nervous system, viz., the ganglion cells.

The preparations are made in the following way: Pieces of the ventral cord one and one-half to three centimeters in length are dissected out of a freshly killed worm in physiological salt solution. These pieces are then stretched in small, wax bottomed dissecting pans and held in a straight position by means of

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Page 1615 small pins at either end and the lateral nerves are laid out in their normal positions. The salt solution is then poured off, leaving the pieces of cord straight and flat against the bottom of the pan. A considerable quantity of vom Rath's fluid (formula given above) is now poured into the pan and the tissue is left in this for two hours for thorough fixation. In a short time (fifteen to twenty minutes) after this fixation has begun the pins may be removed, and the pieces of cord handled freely without danger of distortion. After removal from the fixing fluid the tissue is washed in methyl alcohol; blackened in pyroligneous acid from eight to twelve hours, and washed again in methyl alcohol. The pieces may now be dehydrated in 96 per cent. and absolute alcohol, cleared in cedar oil and embedded in paraffin.

Pieces of the cord from three-fourths to one and one-half centimeters long prepared in the way described are cut into longitudinal sections twenty mikrons thick. The plane of cutting is made as nearly as possible horizontal, i. e., at right angles to the median dorso-ventral plane of the cord. The sections are then stained on the slide by the Heidenhain iron hæmotoxylin method and then differentiated in a two per cent. iron alum solution till the axis cylinder processes alone retain the color. The sections are faintly counterstained with orange G and, after dehydration and clearing, mounted in balsam.

The resulting preparations show the nerve fibers stained an intense black on a light yellow field, and as a result of the great thickness and the plane of cutting of the sections, a single fiber may be followed along the cord for a long distance. For precision and distinctness in the differentiation of fiber elements these preparations leave little to be desired, and the simplicity of the process as compared with other nerve methods is a decided recommendation.

Zoölogical Laboratory, University of Michigan. RAYMOND PEARL.

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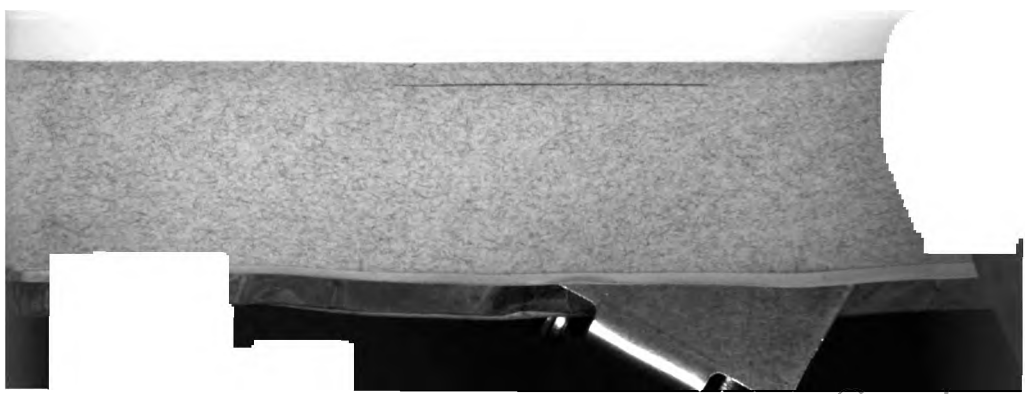
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ON THE PREPARATION AND PRESERVATION OF SOME MARINE ANIMALS AS MUSEUM SPECIMENS.

By H. C. Sorby, LL.D., F.R.S.

THE method adopted in killing the actiniæ in a well-expanded condition was to keep them in sea-water, to which a few crystals of menthol were added. Though very little is dissolved, yet this causes them to dilate well, and in many cases finally kills them in an expanded condition. The difficulty is to know whether they are actually dead, but in no way decomposed, or only stupefied. In the latter case when treated with formalin they contract more or less badly, and hence many may be spoiled; but, if really dead, they contract uniformly to a small extent, owing to the coagulation and hardening of the constituents. The specimens can then be permanently kept in a 4 per cent. solution of formalin, in which, however, some varieties soon lose their natural colour. I have not yet succeeded in preparing satisfactory specimens of *actinoloba* of large size, because the tentacles began to decompose before the body was so dead as not to partially retract them when treated with formalin.

Of the specimens preserved in glycerine there are two series. The first lot, some of which were left at Sheffield in April, were prepared with animals which had been kept for six months in a 4 per cent. solution of formalin, and had to some extent lost their colour. In dealing with these my chief aim was to show the internal



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structure, made visible by the greatly increased transparency. Some of these have now been kept for four months, and, so far, show no deterioration. Now that I am living on my yacht, and can obtain living animals, my aim has been not only to show the internal structure, but to preserve the natural colours. The specimens last sent belong to this series, marked 1 to 9. After not more than two months' experience it is, of course, impossible to decide whether the natural colours will be permanently retained in each particular case; but, so far, the results are very encouraging. In some instances I have kept the same animals in glycerine, in diluted alcohol, and in a 4 per cent. solution of formalin; and, even already, the comparative permanence of the colour of those kept in glycerine has been well demonstrated. In several cases these other liquids soon dissolved out a good deal of the colour, or altered its character by decomposition, whereas, so far, no such changes have occurred in those kept in glycerine. On the contrary, it brings out the colouring, much as occurs when wood is varnished, and does not cause the dull and opaque appearance so often produced by the action of alcohol or formalin. I, however, think it would not be well to keep the specimens in bright daylight.

In a few cases the general result seems better when the animals are kept in a mixture of equal volumes of glycerine and a 4 per cent. solution of formalin.

It is a great advantage to keep some animals for a short time in alcohol or formalin, so as to harden the tissues and prevent undue contraction when transferred to glycerine; and in all cases to first use somewhat diluted glycerine, and finally transfer them to strong. By this means both the form and colour of some worms are well preserved. The whole subject is, however, only in its

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infancy; and so many new facts turn up almost every day, that what I now write may be out of date before it is read.

NOTES ON THE SPECIMENS SENT FOR EXHIBITION.

1 and 2.—Fish showing well the internal structure. The plaice is mounted in a mixture of glycerine and a 4 per cent. solution of formalin.

3 and 4.—The ascidians were killed by menthol, with the apertures well extended, and they show much of the internal structure.

5.—The hermit crab shows well the colour, much of which is soon dissolved out by alcohol or formalin.

6.—The *sabella* shows the colour of the body and plumes much better than when kept in alcohol or formalin, which make the body dull and opaque.

7.—*Syphenculus.* One specimen is in a contracted state, and the others well distended, having been killed by fresh water. All show well the internal structure.

8 and 9.—The star fish show the natural colour to great advantage, and to some extent the internal structure.


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ON THE PREPARATION OF MARINE ANIMALS AND PLANTS AS TRANSPARENT LANTERN SLIDES.

BY

H. C. SORBY, LL.D., F.R.S.,

VICE-PRESIDENT OF UNIVERSITY COLLEGE, SHEFFIELD.

Extracted from the COMMEMORATION VOLUME.


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ON THE PREPARATION OF MARINE ANIMALS AND PLANTS AS TRANSPARENT LANTERN-SLIDES.

By H. C. Sorby, LL.D., F.R.S., &c.

On several occasions I have used a number of my lantern-slides of marine animals in illustrating popular public lectures at our college, but gave only a rough general account of the methods employed in mounting them. It has been thought that a more detailed description would be suitable for this commemoration volume.

It is now somewhat more than a dozen years since I first prepared marine plants as lantern-slides. They were simply mounted dry between two pieces of glass, and when thin and flat showed extremely well. The chief objections to this simple method are that some algæ are too opaque, and unless the slides are kept very dry, mould grows along the surface of the glass. Unfortunately, for many years I was deterred from mounting algæ in Canada balsam by the belief that it would either dissolve out the colouring-matters or destroy them. It was only in the year 1896 that I tried the effect, and, to my surprise, found that it had no injurious result; and by making the plants more transparent they show far better on the screen. Up to the present time there is no indication of any action on the colouring-matters, which are probably protected by substances quite insoluble in balsam. What may be the result when they have been kept for many years, time alone can decide. The preparation of such slides is comparatively simple. Suitable specimens are floated out on the glass in fresh water, and properly arranged with a small brush or a needle fixed on a holder, and then allowed to dry. In many cases they adhere sufficiently well to the glass, but sometimes it is necessary to gum them here and there. The specimens are finally mounted in Canada balsam, as will be described further on.

It is about ten years ago that I first attempted to prepare lantern-slides with marine animals. At first I did not mount them in balsam, but very soon found that this is in almost every case not only desirable but even essential, since they so readily become mouldy, sometimes are attacked by mites, and are often far too opaque. Some also scale off from the glass and break to pieces, unless mounted. The success of the preparations depends almost as much on the proper mounting with balsam as on anything else.

Of course the first thing to be done is to catch your animals. Some are obtained by using a dredge, but quite as many by other means. Some are


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caught in a small beam-trawl, with fine meshes, worked from a two-oared boat. Many are obtained by collecting on the bottom exposed when the tide is down, or by carefully breaking up the mud, or dug out of the sand. Many interesting objects can be obtained by means of brass sieves on a long handle, when they are seen floating near the surface alongside the yacht, or from a boat. Some of the best and rarest specimens have been caught in this manner. Almost every different kind of animal requires a different subsequent treatment. Such as *Cydippe* are almost worthless if caught in a dredge or trawl. They should be caught in a sieve when seen on the surface, and put directly into the preserving liquid. I used to employ methylic alcohol, diluted with half its volume of fresh water, but last year found that it is far better to put them at once into formic aldehyde diluted with nine times its bulk of water, so as to be a 4 p. c. solution of the anhydrous compound. The various species of *Medusæ* are also caught with the sieves, and generally put into a large vase with sea-water to see whether they are satisfactory, and also sometimes kept in it a few hours in order to clean themselves. For these I also have employed the alcohol diluted as named above, but I find that the 4 p. c. solution of formic aldehyde is far superior. Many animals should be kept several days in clean sea-water to clean themselves and to get rid of the sand and mud with which they are often filled. In the case of animals like crabs, large and small specimens must not be kept together, since next day it may be found that the larger have eaten up the smaller. Some kinds of small fish live well in sea-water for weeks or months, whilst other kinds, caught in the same manner, die in a few hours. Certain species of animals vary much according to the locality and time of the year, and also in different years, so that one specimen may be dark and opaque, and another pale and transparent. In some cases there is also a remarkable difference in the development of the blood, as well as in other particulars. The only way to get excellent results is to mount several, and pick out the best, which perhaps cannot be known until the specimens are finally mounted in balsam. I may here say that some of the most interesting animals occur but rarely, and a number of my best are the only specimens of the kind I have ever caught, though living five months a year with marine animals on all sides of me.

Having said what appeared desirable about collecting, I now proceed to describe the chief special methods necessary in mounting, which vary greatly in the case of different animals. Often little else is wanted than to arrange them properly on a lantern-glass, so that they touch it more or less completely all over their under surface, and then to drain and dry them. Many readily adhere round the drying edges, before the central parts are dry; and being thus fixed, they do not shrink laterally on further drying, but merely become thinner. On finally drying completely they may partially scale off, and it may be desirable to gum them down in one or more places, lest they should become loose when mounted in the balsam.

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There are a few animals that will not adhere at all to the glass, and yet shrink greatly. This circumstance has so far prevented me from making satisfactory slides of *Actiniæ*. I have succeeded with every other group.

Few animals are more easy to prepare than small flat fish like soles and dabs, 2 or 2½ inches long. These are killed by putting them into dilute alcohol, and arranged on the glass as soon as dead, whilst still limp. The chief matter is to arrange out the fins neatly. These soon dry, and adhere well; but in order that the side near the glass may keep flat, it is desirable on further drying to adopt a plan which I find most useful in many other cases. Very few, if any, animals will adhere in an objectionable manner to thin paper soaked with bees-wax, and, having laid such over the animal, pressure can be applied. What is wanted is that this pressure should be fairly uniform, and not merely on the thick parts. This is easily done by having a stout lantern-glass covered by two or three thicknesses of fine thin flannel, which is pressed down by a smaller or larger weight, so regulated as not to crush or distort the animal, but rather to retain as much as possible the natural shape and show the internal structure. The animal then dries through this flannel, and at the same time keeps sufficiently flat on the glass. Finally, any specially high parts can be pressed down by using a flat glass without flannel and a heavier weight.

A considerable variety of marine worms can be made into most excellent transparent slides, showing not only their general shape and colour, but also much of their internal structure. *Sabella* may be named as a specially good example. Such animals should be killed by keeping them for a short time in dilute alcohol. The aim should be to dry them before partial decomposition sets in and destroys the small blood-vessels. If possible I dry them on deck, when the weather is fine and breezy, covering them with muslin if wasps are about, which bite pieces out of them very soon. In damp weather a good plan is to dry the specimens in a tin box by means of flannel well dried at a fire, by which means they may be prepared successfully, when otherwise they would be worthless. If all goes on well it is possible to dry and permanently preserve such worms as *Nereis*, so as to show not only the chief blood-vessels but even the smallest branches. What I have done lately proves very clearly that, in some cases, it is a most excellent plan to cover the animals with a thick coating of liquid Canada balsam as soon as they are dry, and to dissolve this off in benzole shortly before mounting them. This system promises well for future work. Varnish is objectionable. If all is properly done the blood may retain its red colour for years without any apparent change.

Some species of *Terebella* can easily be prepared, but it is difficult to extract others from their tubes and to kill them without their distorting themselves greatly. By killing them whilst in their tubes by hot water, and then cutting open the tubes, I have succeeded in mounting specimens so as to show the internal anatomy very well; the digestive organs and chief blood-vessels being very distinct. The tentacles and branchiæ do not

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retain their colour when dried, but by acting on the tentacles, when on the glass, with an alcoholic solution of madder, or an aqueous solution of log-wood, they can be stained so as to be of their natural colour, and the branchiæ may be so treated with carmine as to make the finished specimens like the animals are when alive. It is sometimes almost impossible to disentangle and arrange the numerous long tentacles of some *Terebella* and *Cirratulus*, and at the same time to quickly dry the body. This difficulty may be overcome by cutting off the tentacles at once, keeping them in diluted alcohol, drying the body on the glass, and afterwards mounting the tentacles when stained of the natural colour of the living animal. This system is not only better, but takes less time, since it is much easier to separate the tentacles.

I have not yet succeeded in preparing specimens of *Arenicola* when in their natural condition, so as to show their internal structure well, but have made some most excellent preparations by carefully cutting the animal open from end to end, spreading it out on the glass, and displacing the intestine and its appendage so as to be quite clear of the body, but yet to show the numerous blood-vessels passing to the lateral branchiæ from the main trunks along the intestine. Of their kind no preparations could be more satisfactory than some thus made, since the general anatomy is seen to great perfection, and the colour of the blood has remained unchanged for years, though in other cases, from some unexplained cause, it quickly turned brown.

Priapulus needs a special method. It should be killed by putting it into fresh water, and left in it so long that the body just begins to get limp. It can then be easily arranged on the glass, and adheres fairly well without lateral contraction. If mounted at once or previously kept in alcohol the body is too hard and will not adhere to the glass, and on drying contracts so much laterally as to become very unlike the living animal.

The internal anatomy and general structure of *Priapulus* are best seen by cutting the animal open from end to end, and staining the whole with Beale's carmine or Kleinberg's hæmatoxylin. It is finally spread out on the glass with the internal organs left *in situ* or thrown somewhat on one side. When thus prepared, the muscular structure of the body-wall and the general internal anatomy are seen to great perfection.

Some species of simple and compound Ascidians present no special difficulties, and retain their natural colour very well. Sections of thick-growing compound species show their structures well.

I have prepared many excellent slides with *Synapta*. It is extremely difficult to kill these animals in a fully extended condition, but it may be done fairly well by letting them distend themselves as much as they will in a small quantity of sea-water, and then suddenly pouring them into hot water. They will not, however, half extend themselves in a small bulk of water. It is easy to kill them in a contracted state. When dried on the glass the included sea-water often gives rise to crystals of salt.

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This may be got rid of by coating the animal with a thick solution of clear gum arabic and keeping the whole damp for some days, covered up over a small quantity of alcohol diluted with an equal bulk of water. Of course all gum extending on the glass must be washed off before mounting in balsam. Distended specimens mounted in their natural condition show the general anatomy fairly well, but not so well as when stained with madder or carmine. It is interesting to mount side by side extended and contracted specimens, and another showing the effect of keeping these animals in sea-water to which a most minute quantity of citric acid or fruit juice has been added, which act as most virulent poisons, causing them to contract in the most extraordinary manner. In order to show the complex ovaries to advantage, it is best to cut open the specimen, and stain it. The oviducts full of ova may then be seen.

It was the desire to preserve some of the beautiful Nudibranchs that led me to mount animals on slides. The lovely purple *Eolis* quickly loses its colour in alcohol. It should be killed in dilute alcohol but kept in it a very short time, and then arranged on the glass and nearly dried. A strong solution of gum should then be placed over it, and the whole kept damp over diluted alcohol, to enable the gum to soak well into the animal. When thus treated the purple colour is not dissolved out by the Canada balsam, which otherwise occurs. I have specimens which have been mounted for more than six years without showing any further change than the loss of a bluish tint, which occurs almost at once. Unfortunately some species of Nudibranchs cannot be killed without breaking themselves to pieces.

Crustacea and allied animals, covered with a hard shell, will not adhere to the glass; and large and thick specimens may be too opaque. Still, most excellent transparent slides may be made with the so-called spider crabs, and these sometimes show well the manner in which they are covered with a growth of sertularians, sponges, ascidians, &c. Certain species are very dark and opaque in some localities, but in others, pale and transparent. In some cases I have dissolved out all the carbonate of lime by the action of a little hydrochloric acid in diluted alcohol, but in some localities specimens three inches or so in the spread of the legs are sufficiently soft to render this unnecessary. The animal is properly arranged on the glass, and at first gentle, and afterwards stronger, pressure applied, using waxed paper and glass covered with flannel; by which means the whole may be pressed flat without material distortion. The body and legs may indeed be made a little wider than natural, but this is to a great extent counteracted by lateral shrinking. At all events the general results are extremely good. Thick clear gum is then run under and over the whole, and the specimen kept damp long enough for the gum to penetrate. The excess is finally cleared away from the bare surface of the glass before mounting with balsam. In order to show well the attached parasites, it may be well to slightly stain them when on the glass by means of an alcoholic solution of

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madder. Some of my later slides prepared in this manner are extremely satisfactory, being transparent all over and covered with parasites. *Caprella* on the whole comes out best when previously stained with madder, since the muscles are then seen. In a similar manner excellent slides may be made with *Nymphon*. I found that the best slide of *Idotea* was made with a large specimen which had just changed its skin in one of my aquaria.

Different kinds of starfish require different treatment. Some show well when mounted in their natural state, but others show certain facts better if the carbonate of lime has been dissolved out and the animal stained with carmine so as to be nearly the same colour as when living. Such slides of small specimens of *Solaster* may be prepared so as to show fairly well the pseudopodia and the internal structure. Sand-stars are best killed by suddenly dropping them into strong alcohol, when they do not break themselves to pieces. They are, however, then far too brittle to mount until the carbonate of lime has been dissolved out, when they become limp and tough, but show all the detailed structure.

Various species of Mollusca can be prepared so as to show their general anatomy by dissolving away the shell with hydrochloric acid in diluted alcohol. The organic matter of the shell retains the natural form, and shows the attachment of the various parts of the animal, which may be stained or not according to circumstances. Judging from what I have done, it will be possible to prepare instructive slides from the shells alone of some species, by dissolving away the carbonate of lime and mounting the membranous residue, which tains the natural form and much of the colour.

When first I attempted to mount Medusæ as lantern-slides, I looked upon them as most unpromising, and never imagined that it would be possible to prepare such specimens as I now possess. In the first case I dried one in its natural state and obtained little else than crystals of salt, which rapidly deliquesced. I then saw that the first step must be to dissolve out all the included salt. For some years I used to drop the newly caught specimens of *Aurelia* into methylic alcohol, diluted with half its bulk of fresh water, and after leaving them in for some hours, with occasional movement so as to prevent adhesion to the glass, they were digested over and over again with fresh diluted alcohol, until it was thought that all the salt had been removed. The chief difficulty is to so carry out this process that each member of the surrounding fringe remains separate, and does not adhere to others into a slimy mass. Specimens so prepared are so colourless and transparent that little of the general structure can be seen, but if kept many months in alcohol they turn somewhat brown-yellow and show their structure moderately well. On the whole it is however better to stain them. I have experimented with a great variety of colouring-matters, but find that the best are tincture of madder, Beale's carmine, methyline-blue, port wine, and tincture of galls. My very best specimen was prepared by using port wine, the tannic acid having hardened the fringe. Tincture of galls has a similar effect, and stains the specimens of a yellow-brown colour, and the

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eyes are seen to great advantage. The canal-system and the general structure are well seen when Beale's carmine is used, but the colour is unnaturally bright, whereas the colour of madder is more in harmony with nature. Methyline-blue gives good results with fresh specimens, but does not stain those which have been kept long in alcohol.

Though I have many splendid specimens prepared as described, my last year's experience shows that, at all events for some Medusæ, a 4 per cent. solution of formic aldehyde is far better than alcohol. Into this the newly caught animals were at once put, and it was subsequently used to dissolve out the salt. The superiority of this over alcohol is that Medusæ retain their form almost unaltered, and the most delicate parts can be moved about and arranged without fear of tearing. The only serious objection is that the very delicate fringe of *Aurelia* may be too rigid to be properly extended. No such objection exists in the case of *Cyanæa* or *Chrysaora*; and *Cyanæa* may be stained so as to be of nearly the natural colour, which is otherwise lost. The use of formalin and final staining by carmine or methyline-blue promise very improved results in the future.

In mounting Medusæ some special methods are necessary. Having removed the salt and stained the specimen as thought desirable, the lantern-slide glass is put into one of the usual developing dishes and the animal floated out and to some extent properly arranged, when under the liquid. This is then sucked out with a pipette having an elastic top, and the glass and animal then taken from the dish and further arranged by using a small brush or needle fixed on a small stick. The specimen must then be half an inch thick in the centre. No attempt should be made to dry it at once, since the greater part of the included liquid usually diffuses out, and nearly the whole can be drained off by keeping the slide inclined and covered up so as not to dry. In some cases the liquid passes off badly, but comes off rapidly, if a solution of gum is spread over the animal. The fringe may then be more completely arranged and, when all is satisfactory, drying may commence. As the edge dries it should be covered with a strong clear solution of gum to which a little glycerine has been added to make it less brittle when dry, and this process continued until the whole specimen has been covered. If on drying crystals of salt are formed in the animal, they may be got rid of by allowing them to diffuse into a solution of gum subsequently removed. The specimen should then be kept for some days in a wet state so that the gum may soak well in and any small bubbles not easily removed mechanically may disappear by absorption. At first I used to keep the specimens in a developing dish over a little water, covered up with a closely fitting plate of glass, but sometimes in the course of a single day they became coated with a long growth of mould. If, however, instead of water they are kept over alcohol diluted with an equal volume of water, they may remain wet for weeks, without any such growth of mould or such alteration in the gum as is produced by the action of stronger alcohol. This use of diluted alcohol is of very great service in many cases. It is very desirable


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not to finally dry too quickly, but, as it were, to anneal the specimens; since contraction may give rise to sufficient tension to cause them to crack and scale off from the glass. If this should occur before mounting in balsam, by wetting and gumming, and keeping damp over diluted alcohol, the specimen may be made perfect again. When finally mounted in balsam all such danger ceases. I have been particular in describing all these details since, before I found out how by a little management excellent results could be obtained without any difficulty, I used to be at a very great deal of trouble, and yet the results were very bad.

There is no serious difficulty in mounting *Cyanæa* or *Chrysaora* so as to show a side view, as when the animal is seen swimming, but it is only under special conditions that *Aurelia* can be mounted on its side, so as to be in proper perspective. In order to do this its substance must be in a peculiar plastic condition, which in some cases depends on the strength of the alcohol used in the preparation, but also on other conditions not easy to understand, perhaps partly on the time of the year, or even on individual differences in the animals themselves.

Curious and interesting results can be obtained by staining animals when alive in sea-water. In some cases the effect is not the same as when the animals are dead. Of course a colouring-matter must be used which is not poisonous. The addition of a few chips of logwood turns the water to a good brown, and, as far as I have been able to judge, animals live longer in it than in normal sea-water. Methyline-blue also answers very well, and sand-stars so stained, and the carbonate of lime afterwards dissolved out, show their anatomical structure surprisingly well. In some cases a small quantity of Beale's carmine gives good results, and brings out sundry facts well. Strange to say the coloured juices of fruits, such as bilberries, elderberries, and plum, which one might fancy would be very harmless, turned out to act as violent poisons on some animals.

I must now consider cases in which it is desirable to get rid of part of the colouring-matter, either natural or developed on keeping. Some animals contain a substance which in time turns brown. If when alive the animal is nearly colourless, and afterwards turns pale brown, this may be an advantage, and answer the purpose of a stain. There are, however, cases in which the preparation becomes so dark as to show on the screen merely as a black uniform shadow. Specimens of *Sepiola* are examples of this bad result. After many trials I have overcome the difficulty. This animal should be allowed to die naturally in sea-water, when usually the pigment-cells are so contracted as to show only as very small separate spots. The specimen should then be arranged in a suitable manner and dried, when by using gentle pressure it becomes comparatively thin, and yet not materially distorted. The eyes can also be made to show well. After having been almost dried, it should be kept for some weeks in diluted sulphurous acid, in which it does not decompose, but which dissolves out the objectionable constituents, without injuring the natural spots, the ink-bag, or he colouring sometimes seen in certain internal organs. When again mounted

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on glass, it may be desirable to remove the skin from one side, so that the excess of dark spots may not hide the internal structure. When all has been properly managed, the final result is most excellent, and much of the internal anatomy can be seen ; which may vary considerably in different specimens. Diluted sulphurous acid is also most useful for other purposes. Specimens of *Aphrodite* two inches long are naturally much too thick and opaque, but after keeping in the acid become sufficiently thin and transparent to make excellent lantern-slides. I have also found it answer very well in preparing *Lepas* and *Conchoderma*, from which it dissolves the calcareous shells and leaves the animals transparent and of fine purple colour. The most remarkable results can be obtained with small fishes. If plaice about 2¼ inches long are kept in alcohol and then for a few weeks in diluted sulphurous acid, the earthy matter of the bones is dissolved out and only cartilage left: the general colour is reduced, and the thickness diminished ; but, strange to say, the arteries and enclosed blood are so little altered, that when the specimens are mounted the aorta and branching arteries are well seen over the whole animal. The development of the arteries is much less in some other fishes, but this method of treatment makes them so transparent, that their general structure comes out extremely well on the screen. Many small fishes are, however, naturally quite sufficiently transparent when balsam has well penetrated into their tissues.

Having duly prepared the dried animals, it may not be convenient to mount them at once in Canada balsam. They may then be kept in tin boxes with flannel which has been well dried at a fire, so as to absorb any moisture that may be in the air. When thus kept, even for many months, they usually do not undergo any sensible change, and do not go mouldy, which otherwise they do very readily, especially when out at sea in a yacht.

I must now conclude by describing the methods employed when finally mounting the specimens in Canada balsam. At the four corners of the glass should be gummed small pieces of blackened cardboard, of such a thickness that the cover-glass will just clear the object and not rock. The glass with the animal should be kept for a short or longer time in benzole ; and, in the meanwhile, the cover-glass should be warmed on a suitable stand over a small burner, and a fair quantity of liquid balsam placed in the centre. It need not be kept hot for more than a few minutes, so as to allow the bubbles to rise to the surface. The glass with the animal is then taken out of the benzole, and carefully placed over the balsam, so as to catch up as few bubbles as possible, the benzole causing the greater part to burst and disappear. If too little balsam has been used, more is easily run in between the glasses ; and if only a few bubbles have been caught up they soon disappear. If there are more than desirable, they can be got rid of by keeping the slide slightly inclined until they rise to one edge and can be removed. After keeping cold for a few days, for the balsam to harden at the edges, it is on the whole convenient to gum narrow slips of thin paper in the centre of each side, so as to hold the cover in place, and make it possible to clean

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off the superfluous balsam, afterwards varnishing the strips. It is best to let the slide remain horizontal for some days, or even weeks, for the balsam to get hard at the edge, and then to bind with thin gummed paper two opposite sides, which should be allowed to dry before the other two are gummed on, since in drying the paper may contract and squeeze out some of the balsam. When all four are fixed and dry, they must be varnished, since otherwise they will become wet, relax, and draw in air, when the final binding is done. After the varnish is dry, the whole slide must be very carefully bound all round with black paper, which should be *glued* on thoroughly well. I need not describe all the needful precautions, which will occur to any one carrying out this part of the work. The importance of very completely sealing up the balsam, by binding the edges, will be apparent when it is borne in mind that it will remain liquid, and, when the slide is put into the lantern, will get more or less hot and expand, and would readily find its way out through even a small leak. I have, however, had scarcely any trouble from this cause, since I have so completely boxed it in that the glasses or binding yield sufficiently. I may also say that though many of my slides have now been made for more than six years, I have not observed any deterioration, but, on the contrary, many have greatly improved owing to the balsam having more completely penetrated into the tissues, and the included air disappeared. When this has taken place, the specimens are far more transparent and show their structure far better than the living or dead animals. Nearly all my slides have been kept in the dark, and even those 6 or 8 years old are as good as ever. Some have been kept about the same time in a strong light at the Sheffield Public Museum, Weston Park, and, as far as can be judged, have not faded, even when imperfectly mounted. So far there is thus strong probability that they will remain in a satisfactory state for many years.

The accompanying collotype plate (Plate VII.) will to some extent show the character of the mounted specimens. It has been taken from photographs of the slides, and though the general results are fairly good, the minute detail so well seen on magnifying the photographs is to a large extent lost. In all cases the size is somewhat less than the real animals. These in some cases are yellow, and photograph so dark as to conceal structures visible in the specimens themselves.

Fig. 1. *Sabella.*

Fig. 2. *Priapulus*, showing the intestine fairly well.

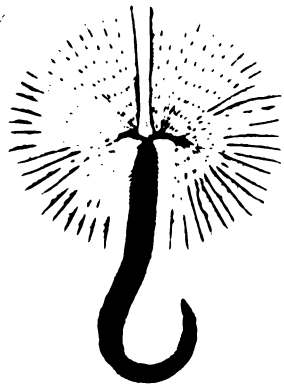
Fig. 3. *Arenicola*, cut open, spread out, and the digestive organs thrown on one side. The structures of the body-wall, the principal nerve, the viscera and blood vessels fairly well shown.

Fig. 4. *Aurelia* stained with carmine.

Fig. 5. *Ophiura* kept alive in sea-water with methyline-blue, then suddenly killed by strong spirit, and the carbonate of lime removed by dilute hydrochloric acid. The structures well seen in the original, but some important detail lost in the collotype.

Fig. 6. Plaice kept in alcohol and then in dilute sulphurous acid, so as to remove the bones but to leave the aorta and arteries filled with blood. The larger are quite distinct, but the smaller lost in the collotype.

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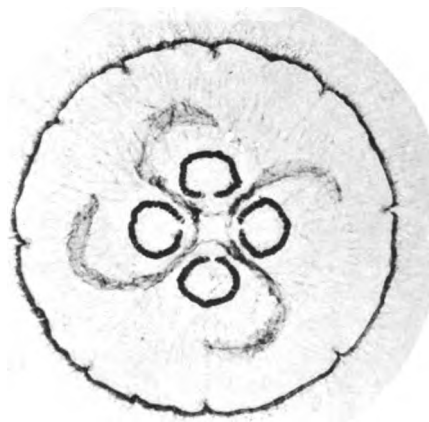




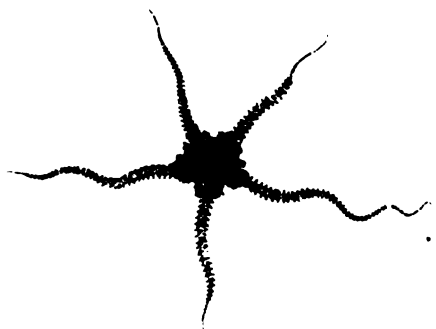
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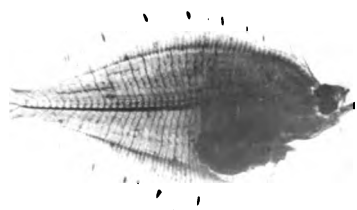
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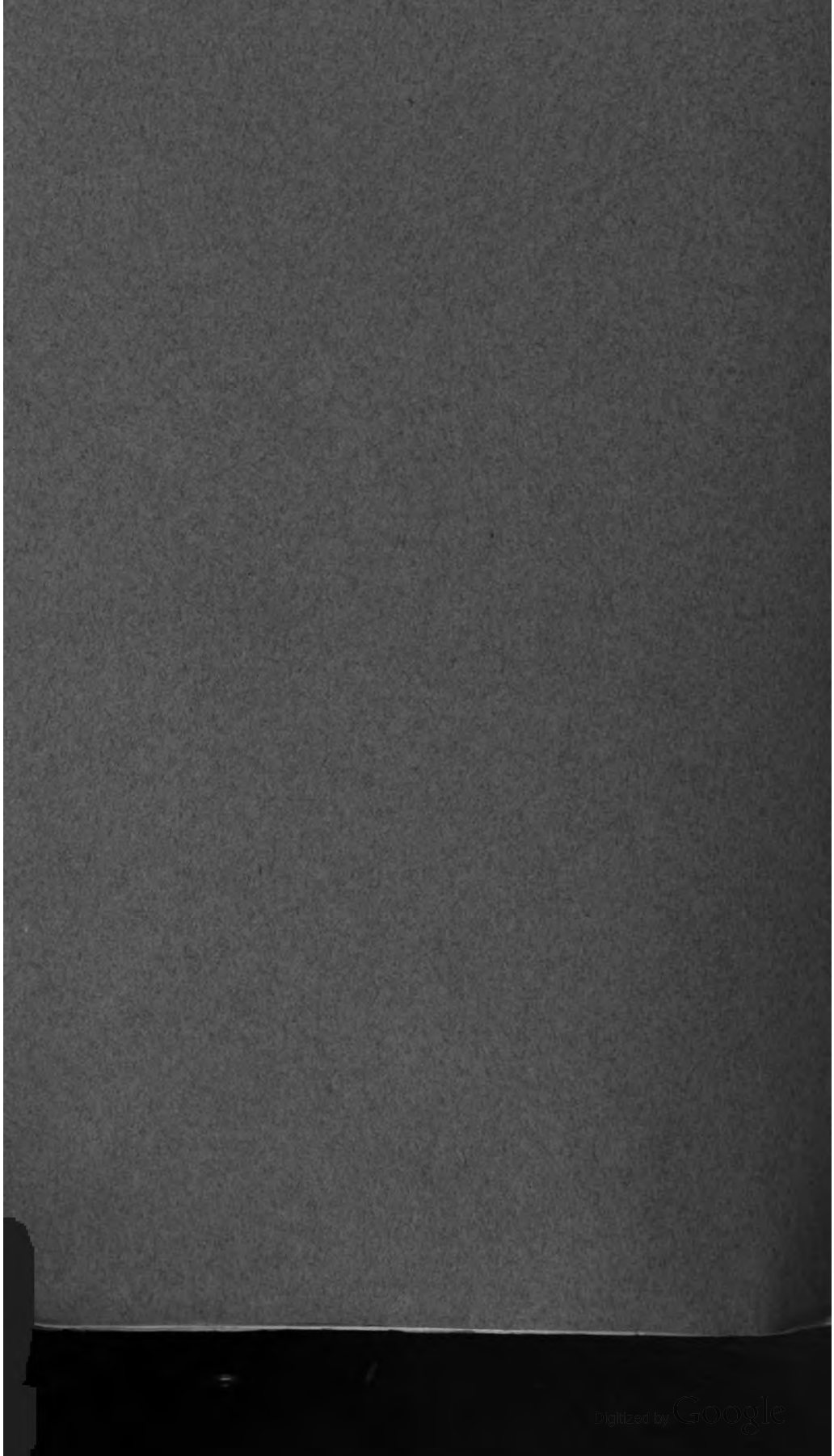

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